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Bench to Bedside: Understanding Symptom Response to Acupuncture Treatment and Designing a Successful Acupuncture Treatment Program

PRINCIPAL INVESTIGATOR:
Lisa Conboy, MA, MS, ScD

CONTRACTING ORGANIZATION:
MCPHS University
Yadelyn Mahoney
170 Longwood Ave
Boston, MA 02115-5804

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Lisa Conboy, MA, MA, ScD

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E-Mail: lisa.conboy@mcphs.edu

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
MCPHS University
Yadelyn Mahoney
179 Longwood Ave
Boston, MA 02115-5804

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14. ABSTRACT

This secondary data analysis project used data from our pragmatic Randomized Controlled Trial considering if acupuncture is effective in the treatment of Gulf War Illness. We achieved our two aims of completing scale construction for remaining psychosocial and clinical measures, and determining the relationships between dose of acupuncture and effect on secondary outcomes in this sample. These analyses allow us to move deeper into a better understanding of GWI. In addition to the 14 professional conference presentations listed in the **products** section (within), we completed 6 papers which have furthered our understanding of GWI in ways relevant to different users: scientists and clinicians working with GWI, acupuncturists working with a veteran population, veterans.

15. SUBJECT TERMS
Acupuncture, Gulf War Illness, Complex Medical Illness

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INTRODUCTION: In 2009, our study team was awarded a three-year Congressionally Directed Medical Research Program grant to implement the RCT "The Effectiveness of Acupuncture in the Treatment of Gulf War Illness" (W81XWH-09-2-0064). We successfully completed this project in January of 2013 with positive results of clinically and statistically significant improvement in pain and physical function[i] . This current application, "Bench to Bedside: Understanding Symptom Response to Acupuncture Treatment and Designing a Successful Acupuncture Treatment Program", originally had 5 Aims. Of the 5 Aims, two of these were recommended for funding: Aim 2: Complete scale construction for remaining psychosocial and clinical measures, Aim 3: Determine the relationships between dose of acupuncture and effect on secondary outcomes in this sample. These aims support two objectives utilizing data from the parent grant: 1) Better define and describe this complex disease and how healing may happen using acupuncture, 2) Report on the best acupuncture protocols for GWI veterans and specific GWI presentations.

KEYWORDS: Acupuncture, Gulf War Illness, Complex Medical Illness.

ACCOMPLISHMENTS:
What were the major goals of the project?
Aim 2: Complete scale construction for remaining psychosocial and clinical measures, Aim 3: Determine the relationships between dose of acupuncture and effect on secondary outcomes in this sample. Both of these Aims have been completed as per the Statement of Work (Appendix B), with associated products described below and attached in Appendix A.

As per the SOW for Aim 2 we trained the research assistants, and reviewed goals with consultants in month 1 and 2. We completed IRB approval through the New England Institutional Review Board by month 3 and HRPO approval by month 6. Data cleaning continued through month 8.

Toward the execution of Aim 3 In month nine Dr Conboy began data analysis of predictors of clinical improvement with the help of Dr. Locascio. Dr Conboy worked with Dr Locascio through the complex data set to eventually determine 5 strong baseline factors that can be used for symptom change prediction. This process was time costly due to missing data, and the data structure requirements of factor analysis. Along the way we learned that the Chinese Medicine information will be difficult to integrate into the main data set. At this point only a poster describing the process has been produced. Drs Conboy and Taylor-Swanson will continue to take this work forward.

Throughout the entire grant, Dr. Conboy convened phone meetings with the the consultants approximately every 6 weeks. Email was used frequently for some simpler questions. At these meetings present were Drs Bell, Goldstein, Schnyer, Taylor-Swanson, and Conboy, as well as Joe Chang. Kai-Yin Hsu was also present at most of these planning meetings. At these meetings we initially shared relevant literature, then reviewed analyses plans, and assigned/volunteered for aspects of manuscript preparation.

Manuscript preparation began at 18 months, and the resulting products are listed under the PRODUCTS section below.

What was accomplished under these goals?
Our parent trial offered evidence that acupuncture can be effective in treating the symptoms of Gulf War Illness. This present grant allowed us to move deeper into a better understanding of GWI. In addition to the 14 professional conference presentations listed in the **products** section below, we were able to complete multiple papers (*Appendix A)* which have furthered our understanding of GWI in ways relevant to different users. First to scientists and clinicians working with GWI, our publication *Characteristics of Gulf War Illness participants in an acupuncture study* tabulates and describes our baseline sample description to offer a rich description of our sample; we also compare levels of symptom expression to normative values for reader's ease.

Second, our publication *Effectiveness in Clinical Trial Recruitment: An Analysis of Methods used in a Novel Randomized Clinical Trial for the Treatment of Gulf War Illness* describes our multi-modal, successful recruitment process in a manner detailed enough to assist other scientists in studying this population.

Third, of interest to clinicians and scientists more generally are the papers: 1) *Screening for novel central nervous system biomarkers in veterans with Gulf War Illness,* in which a novel biomarker for GWI is proposed via study of our collected blood samples, and 2) *Development of Therapeutic Alliance in Acupuncture Treatments in a Veteran Population* in which we duplicated a common finding in psychotherapy that the quality of patient-practitioner relationship is related to therapeutic outcomes; this finding underscores the importance of relationship rapport in medical interactions.

Fourth, two other publications are of additional interest to the acupuncture community as they treat this complex medical illness: 1) *Matrix analysis of traditional Chinese medicine differential diagnoses in Gulf War Illness,* richly describes the clinical presentations found in our sample in order to familiarize practitioners who want to treat a GWI population, and 2) *The Importance and Determination of Dosage in Acupuncture Treatment: The case of Gulf War Illness,* is one of the few papers with data looking at actual dosage of acupuncture using clinical data, and suggests that higher treatment frequency can offer greater symptom reduction.

Lastly, Drs Conboy, and Joe Locascio, are working with the input of other team members on other ways to model the relationship between dose of acupuncture and effect on secondary outcomes in the sample. At this point we have validated a 5 factor structure of baseline characteristics using confirmatory factor analysis. The complexity of the data, and amount of missing data, made exploratory factor analysis unstable. Our confirmed 5 factors are *pain, sleep quality, psychological health, social context, physical function.* We completed and submitted for review a simpler analysis of this relationship in *The Importance and Determination of Dosage in Acupuncture Treatment: The case of Gulf War Illness* (Appendix A), but will continue to explore the data.

What opportunities for training and professional development has the project provided?
Information has been provided to the acupuncture community describing our study and mode of treatment. This article was published in the trade publication *Acupuncture Today*, is listed below and provided in Appendix A.

How were the results disseminated to communities of interest?
In addition to published articles and conference proceedings mentioned below, our parent paper results were posted to **five** Facebook social media groups that support veterans, Gulf War Illness specific information, or acupuncture: 1) *Acupuncturists on Facebook* (readership 11,958), 2) *Acupuncture researcher share group* (readership 8,125), 3) *Gulf War Illnesses Facebook Group* (**closed group/invitation only,** readership 11,253), 4) *Gulf War Illness-Save Our Service Members Facebook Group* (**closed group/invitation only,** readership 4,965), 5) *Gulf War Illness Veterans Support Group* (**closed group/invitation only,** readership 280).

Parent study results were also posted twice to the Gulf War Illness information cite *91 outcomes* 2015, 2017. (http://www.91outcomes.com/). 91outcomes.com is a health and news website for veterans of the 1991 Gulf War.

In addition, *our study team informally shared our results with Veteran Administration contacts (e.g. Stephanie Taylor PhD, Associate Director, VA HSR&D Center for the Study of Healthcare Innovation, Implementation & Policy, VA Greater Los Angeles Healthcare System)*, other veteran researchers (e.g. *Kim Sullivan PhD at Boston University's CDMRP-funded Gulf War Illness Consortium*), and the veteran advocacy groups (e.g. *Campaign for Military Families*).

What do you plan to do during the next reporting period to accomplish the goals?
Nothing to Report/Final Report

IMPACT:
What was the impact on the development of the principal discipline(s) of the project?
Our results will assist clinicians, VA officials, and veterans in determining if and how acupuncture can be used to treat GWI and other veteran illnesses. Further, the collaborative work using our blood samples assists in a better understanding of the biology of GWI.

What was the impact on other disciplines?
Our results will assist acupuncturists and other Mind-Body practitioners in determining if and how acupuncture and other Mind-Body therapies can be used to treat GWI and other veteran illnesses.

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Our results are applicable to the unfolding integration of complementary medical practices into regular medicine. It will assist acupuncturists and VA officials in determining if and how

acupuncture is used to treat GWI and other veteran illnesses. Our interdisciplinary team has produced products suitable for inter-professional education efforts in medicine, as well as a better understanding of the application of Mind-Body medicines in the treatment of veteran concerns more generally.

CHANGES/PROBLEMS:
Nothing to Report

PRODUCTS:
Publications, conference papers, and presentations
Journal publications. *All listed show acknowledgement of federal support.*

Screening for novel central nervous system biomarkers in veterans with Gulf War Illness.
Abou-Donia MB, Conboy LA, Kokkotou E, Jacobson E, Elmasry EM, Elkafrawy P, Neely M, Bass CR, Sullivan K. Neurotoxicol Teratol. 2017 May;61:36-46. doi: 10.1016/j.ntt.2017.03.002. Epub 2017 Mar 9. PMID:28286177. Published.

Treating Gulf War Illness: The Lasting Effects of Desert Shield/Storm. Joe C. Chang, MAOM, Dipl. OM, LAc, Rosa N. Schyner, DAOM, CFMP, LAc and Lisa Conboy, ScD. Acupuncture Today. 18(8). Published

Characteristics of Gulf War Illness participants in an acupuncture study. PLoS One. Lisa Taylor-Swanson, Kai-Yin Hsu, Joe Chang, Rosa Schnyer, Kai-Yin Hsu, Beth Ann Schmitt, Lisa Conboy. Submitted.

Effectiveness in Clinical Trial Recruitment: An Analysis of Methods used in a Novel Randomized Clinical Trial for the Treatment of Gulf War Illness. Controlled Clinical Trials. Matthew J. Hadfield, OMS-IV, Ann Barbetti MAc, Lisa Conboy, ScD. Submitted.

Matrix analysis of traditional Chinese medicine differential diagnoses in Gulf War Illness. Journal of Alternative and Complementary Medicine. Lisa Taylor-Swanson, Joe Chang, Rosa Schnyer, Kai-Yin Hsu, Beth Ann Schmitt, Lisa Conboy. Submitted.

The Importance and Determination of Dosage in Acupuncture Treatment: The case of Gulf War Illness. Beth Sangree LicAc MAc, Lisa Conboy ScD. American Journal of Chinese Medicine. Under Review.

Development of Therapeutic Alliance in Acupuncture Treatments in a Veteran Population. Saadat Bagherigaleh, MD, Beth Ann Schmitt MEd, MAOM, Lisa Conboy MA MS ScD. PLoS One. Under Review.

Books or other non-periodical, one-time publications.
None.

Other publications, conference papers, and presentations.
2014:

Management of Gulf War Syndrome Symptoms with Acupuncture: Findings of a Wait-list Controlled RCT. Lisa Conboy MA MS ScD, Kai Yin Hsu Lic Ac, Joe Chang LicAc, Lisa Taylor-Swanson Lic Ac, Iris Bell MD, Marc Goldstein MD, Rosa Schnyer DAOM. Oral Presentation. Society for Acupuncture Research International Symposium, Beijing China. April 14-16 2014.

Correlates of Symptom Response to Acupuncture: the Case of Gulf War Syndrome. Poster Presentation. Lisa Conboy MA MS ScD, Kai Yin Hsu Lic Ac, Joe Chang LicAc, Lisa Taylor-Swanson Lic Ac, Iris Bell MD, Marc Goldstein MD, Rosa Schnyer DAOM. Society for Acupuncture Research International Symposium, Beijing China. April 14-16 2014.

Social changes associated with use of Acupuncture Treatment. Poster Presentation. Lisa Conboy ScD & Kate Frame, LicAc. Society for Acupuncture Research International Symposium, Beijing China. April 14-16 2014.*

Management of Gulf War Syndrome Symptoms with Acupuncture: Findings of a Wait-list Controlled RCT. Lisa Conboy ScD. Oral Presentation 2014 International Research Congress on Integrative Medicine and Health in Miami, Florida, USA, May 13 – 16, 2014.

Veterans' Responses to an Acupuncture Treatment RCT. Poster Presentation. Lisa Conboy ScD & Meredith St John Lic Ac. MAOM. Harvard Medical School/Osher Integrative Medicine Research Forum. November 12 & 13 2016 Boston MA.

2015:

How TCM Practitioners Treat Gulf War Illness; findings of an RCT with individualized treatments. Lisa Conboy MA MS ScD, Kai Yin Hsu Lic Ac, Joe Chang LicAc, Lisa Taylor-Swanson Lic Ac, Iris Bell MD, Marc Goldstein MD, Rosa Schnyer DAOM. Poster presentation. Society for Acupuncture Research International Symposium. November 12-14, 2015, Boston MA.*

Improvements in Perceived Social Support and Health Behaviors following Acupuncture Treatment in a Veteran Population. Poster presentation: Lisa Conboy ScD & Christine Cronin DAOM. Society for Acupuncture Research International Symposium. November 12-14, 2015, Boston MA.

A Case Study of Gulf War Illness in a Woman. Poster Presentation. Lisa Taylor-Swanson Lic Ac, Joe Chang LicAc, Iris Bell MD, Marc Goldstein MD, Rosa Schnyer DAOM, Conboy MA MS ScD. Society for Acupuncture Research International Symposium. November 12-14, 2015, Boston MA.

2016

Psychosocial Improvements Associated with Acupuncture Treatments in a Sample of Veterans with Gulf War Illness. Poster Presentation. Lisa Conboy MA MS ScD , Christine Cronin DAOM, LAc. UCSD and VA Military and Veteran Resiliency Medicine Summit, Naval Yard. February 25 & 26, 2016. San Diego, CA.

Using Acupuncture to Treat Complex Veteran Illness. Joe Chang LicAc, Lisa Taylor-Swanson Lic Ac, Rosa Schnyer DAOM, Lisa Conboy MA MS ScD. Poster Presentation. Harvard Medical School/Osher Integrative Medicine Research Forum. November 18 & 19 2016 Boston MA.*

2017

How TCM Practitioners Treat Gulf War Illness; findings of an RCT with individualized treatments. Poster Presentation. Joe Chang LicAc, Lisa Taylor-Swanson Lic Ac, Rosa Schnyer DAOM, Lisa Conboy MA MS ScD. Society for Acupuncture Research International Symposium. April 27-29, 2107, San Francisco, CA.*

Treating Complex Veteran Illness with Acupuncture in the Community. Oral Presentation. Lisa Conboy MA MS ScD, Kai Yin Hsu Lic Ac, Joe Chang LicAc, Lisa Taylor-Swanson Lic Ac, Iris Bell MD, Marc Goldstein MD, Rosa Schnyer DAOM. Society for Acupuncture Research International Symposium April 27-29, 2107, San Francisco, CA.

Development of Therapeutic Alliance in Acupuncture Treatments in a Veteran Population Poster Presentation. Saadat Bagherigaleh, MD, Lisa Conboy MA MS ScD. Society for Acupuncture Research International Symposium April 27-29, 2107, San Francisco, CA.*

Management of Gulf War Syndrome Symptoms with Acupuncture: Findings of a Wait-list Controlled RCT. Lisa Conboy MA MS ScD. Oral Presentation to Center for Healthcare Organization and Implementation Research (CHOIR), June 9, 2017. Jamaica Plain VA Campus Boston, MA.

Website(s) or other Internet site(s):
Posted to Facebook social media groups that support veterans, Gulf War Illness specific information, or acupuncture: 1) *Acupuncturists on Facebook* (readership 11,958), 2) *Acupuncture researcher share group* (readership 8,125), 3) *Gulf War Illnesses Facebook Group* (readership 11,253), 4) *Gulf War Illness-Save Our Service Members Facebook Group* (readership 4,965), 5) *Gulf War Illness Veterans Support Group* (readership 280).

Posted twice to the Gulf War Illness information cite *91 outcomes* 2015, 2017. (http://www.91outcomes.com/). 91outcomes.com is a health and news website for veterans of the 1991 Gulf War.

Informally shared with Veteran Administration contacts and other veteran researchers.

Technologies or techniques
None.

Inventions, patent applications, and/or licenses
None.

Other Products
None

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?

Name:	*Lisa Conboy*
Project Role:	Principle Investigator
Researcher Identifier (e.g. ORCID ID):	ORCID # 0000-0003-2218-7841
Nearest person month worked:	2.4
Contribution to Project:	Dr. Lisa Conboy has acted as the Principal Investigator on this project. She is the main statistician, completing data cleaning and scale construction of the biomedical survey data. Dr. Conboy has conducted regular meeting with consultants, co-investigator and research assistants on the project.
Funding Support:	N/A

Name:	Meredith St. John
Project Role:	*Treatment Specialist*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1*
Contribution to Project:	Meredith St. John has acted as Treatment Specialist for this project. She has reviewed acupuncture specific data from research assistant and consultants.
Funding Support:	*N/A*

Name:	Kai-Yin Hsu
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	ORCID # 0000-0002-5062-9953
Nearest person month worked:	3.6
Contribution to Project:	Kai-Yin Hsu is the Research Assistant for this project. She has coded and organized acupuncture specific data. She has participated in regular meetings with her PI and consultants.
Funding Support:	N/A

Name:	Dr. Joseph Locascio
Project Role:	Biostatistician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Joseph Locascio was the Biostatistician for the project. He has provided statistical consultation and analysis for the project.
Funding Support:	N/A

Name:	Rosa Schnyer
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	0000-0002-6233-5661
Nearest person month worked:	1
Contribution to Project:	Rosa Schnyer was lead consultant on the project. She participated in hypothesis generation and manuscript preparation.
Funding Support:	N/A

Name:	Joe Chang
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Joe Chang assisted with categorizing acupuncture protocols and manuscript preparation.
Funding Support:	N/A

Name:	Dr. Marc Goldstein
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Goldstein used his skill as a medical doctor for secondary data analysis of the population and for manuscript preparation.
Funding Support:	N/A

Name:	Iris Bell, MD
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Dr Bell categorized study data for modeling, participated in hypothesis generation, and manuscript preparation.
Funding Support:	N/A

Name:	Marija Hamed Linjacki
Project Role:	Consultant/Statistician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Ms Linjacki provided statistical analysis of data.
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

What other organizations were involved as partners?
Organization Name:
Massachusetts General Hospital

Location of Organization: *(if foreign location list country)*
55 Fruit St. Boston, MA, USA
Partner's contribution to the project *(identify one or more)*
Massachusetts General Hospital was a subcontract on the grant. They allowed Dr. Joseph Locascio to provide biostatistical consultation and analysis for the project.

Financial support;
Not applicable
In-kind support
Not applicable
Facilities
Not applicable
Collaboration
Massachusetts General Hospital allowed Dr. Joseph Locascio to collaborate on this project.
Personnel
Not applicable
Other.
Not applicable

SPECIAL REPORTING REQUIREMENTS
COLLABORATIVE AWARDS:
None

QUAD CHARTS:
None

APPENDICES:
Appendices A: Publications
Appendices B: Statement of Work
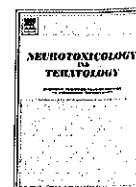Appendices C: Bibliography

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[i] Conboy L, Gerke T, Hsu KY, St John M, Goldstein M, Schnyer R. (2016).The Effectiveness of Individualized Acupuncture Protocols in the Treatment of Gulf War Illness: A Pragmatic Randomized Clinical Trial. PLoS One. Mar 31;11(3):e0149161.
doi: 10.1371/journal.pone.0149161. PMID: 27031099.

Appendix A

Publications

ELSEVIER

Full length article

Screening for novel central nervous system biomarkers in veterans with Gulf War Illness

Mohamed B. Abou-Donia [a,*], Lisa A. Conboy [b], Efi Kokkotou [c], Eric Jacobson [d,i], Eman M. Elmasry [e], Passent Elkafrawy [f], Megan Neely [g], Cameron R. 'Dale' Bass [h], Kimberly Sullivan [h]

[a] Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC, United States
[b] Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States
[c] Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States
[d] Department of Global Health and Social Development, Harvard Medical School, United States
[e] Department of Microbiology, Zagazig University, Zagazig, Egypt
[f] Department of Math and Computer Science, Menoufia University, Shebin ElKom, Egypt
[g] Department of Biostatistics & Bioinformatics, Duke University Medical Center, United States
[h] Biomedical Engineering Department, Duke University, United States
[i] Department of Environmental Health, Boston University School of Public Health, Boston, MA, United States

ARTICLE INFO

ABSTRACT

Gulf War illness (GWI) is primarily diagnosed by symptom report; objective biomarkers are needed that distinguish those with GWI. Prior chemical exposures during deployment have been associated in epidemiologic studies with altered central nervous system functioning in veterans with GWI. Previous studies from our group have demonstrated the presence of autoantibodies to essential neuronal and glial proteins in patients with brain injury and autoantibodies have been identified as candidate objective markers that may distinguish GWI. Here, we screened the serum of 20 veterans with GWI and 10 non-veteran symptomatic (low back pain) controls for the presence of such autoantibodies using Western blot analysis against the following proteins: neurofilament triplet proteins (NFP), tubulin, microtubule associated tau proteins (Tau), microtubule associated protein-2 (MAP-2), myelin basic protein (MBP), myelin associated glycoprotein (MAG), glial fibrillary acidic protein (GFAP), calcium-calmodulin kinase II (CaMKII) and glial S-100B protein. Serum reactivity was measured as arbitrary chemiluminescence units. As a group, veterans with GWI had statistically significantly higher levels of autoantibody reactivity in all proteins examined except S-100B. Fold increase of the cases relative to controls in descending order were: CaMKII 9.27, GFAP 6.60, Tau 4.83, Tubulin 4.41, MAG 3.60, MBP 2.50, NFP 2.45, MAP-2 2.30, S-100B 1.03. These results confirm the continuing presence of neuronal injury/gliosis in these veterans and are in agreement with the recent reports indicating that 25 years after the war, the health of veterans with GWI is not improving and may be getting worse. Such serum autoantibodies may prove useful as biomarkers of GWI, upon validation of the findings using larger cohorts.

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1. Introduction

Approximately one third of the 697,000 US military personnel who served in the Gulf War (GW) from August 1990 to June 1991, have reported persistent symptoms for many years after the war (RAC, 2008; IOM, 2012, RAC, 2016; White et al., 2016). This complex of symptoms, known as Gulf War Illness (GWI), include memory and attention problems, profound fatigue, chronic muscle and joint pain, severe headaches,

persistent diarrhea, respiratory difficulties and skin rashes. GWI is primarily diagnosed by symptom report and no validated objective diagnostic biomarkers currently exist that fully segregate cases from controls. This study was designed to identify objective central nervous system (CNS) biomarkers of GWI using clues from prior clinical studies with GW veterans and from animal studies that modeled chemical exposures experienced by GW veterans.

Clinical studies have reported impaired cognitive functioning and reduced MRI volume and altered white matter microstructural integrity in organophosphate (OP) pesticide, sarin nerve agent and pyridogstigmine bromide (PB) anti-nerve gas pill-exposed GW veteran cohorts (White et al., 2016; Sullivan et al., 2013; Chao et al., 2010;

* Corresponding author at: Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, United States.
E-mail address: donia@duke.edu (M.B. Abou-Donia).

Heaton et al., 2007; Proctor et al., 2006; Sullivan et al., 2003). Animal studies demonstrated that exposure to higher doses of the prophylaxis pill pyridostigmine bromide (PB), the insect repellent, DEET, and the insecticide permethrin and/or chlorpyrifos led to significant brain damage in animal models of GWI (Abou-Donia et al., 1996a,b). Further studies using 60 days of subchronic dermal exposure to DEET and permethrin, alone or in combination, at dose levels approximately equivalent to the exposures that occurred during the Gulf War in a rat-model of GWI, caused the following: (1) a diffuse neuronal cell death in the motor cortex, the different subfields of the hippocampal formation, and the Purkinje cell layer of the cerebellum, accompanied by sensorimotor deficits; (2) significant reduction of MAP-2-positive immunoreactive structures indicating atypical expression of MAP-2 in dendrites of surviving neurons, within the cerebral cortex and the hippocampus that was characterized by a beaded, disrupted, or wavy appearance; (3) a significant upregulation of GFAP-positive expression in structures in the CA3 subfield of the hippocampus, the motor cortex and the dentate gyrus (Abdel-Rahman et al., 2001, 2002a,b, 2004a,b; Abou-Donia et al., 2000, 2001, 2002, 2004; Terry et al., 2003). Similar results were exhibited in animals treated with sarin alone or accompanied by cited-above chemicals, with and without stress (Abdel-Rahman et al., 2004a).

The cytoarchitecture of the CNS is maintained by a complex cellular milieu that involves neuronal and glial cells that must maintain proper communication in order to function properly (Abou-Donia and Lapadula, 1990; McMurray, 2000). CaMKII phosphorylates cytoskeletal proteins, such as MAP-2, tau and tubulin. CaMKII accounts for 12% of all proteins in the brain. CaMKII has the ability to coordinate and transduce upstream Ca and reactive oxygen species (ROS) signals into physiological and pathophysiological downstream responses in the nervous system and cardiovascular biology and disease (Abou-Donia, 1995; Erickson et al., 2011). Tubulin, the major component of microtubules, is responsible for axonal migration and longitudinal growth and is involved in axonal transport. Although tubulin is present in virtually all eukaryotic cells, the most abundant source is the vertebrate brain, where it consists of approximately 10–20% of its total soluble protein (McMurray, 2000). Microtubule-Associated Protein-2 (MAP-2) is found in dendritic compartments of neurons. A loss of MAP-2, is a reliable indication of irreversible neuropathology and is a sensitive marker of seizure-related brain damage (Ballough et al., 1995). Tau Protein, a normal axonal protein, is involved in stabilization and assembly of axonal microtubules. Levels of tau proteins are elevated in the cerebrospinal fluid (CSF) and serum following TBI (Liliang et al., 2011) and has been used for diagnosis of Alzheimer's disease. Myelin basic protein (MBP) is an abundant myelin membrane proteolipid produced by oligodendroglia in the CNS and Schwann cells in PNS and may confirm the clinical assessment of neurodegenerative disorders such as multiple sclerosis and stroke (Jauch et al., 2006). Myelin Associated Glycoprotein (MAG) is selectively localized in periaxonal Schwann cell and oligodendroglial membranes of myelin sheaths, suggesting that it functions in glia–axon interactions in both the PNS and CNS (Schachner and Bartsch, 2000). Glial fibrillary acidic protein (GFAP) is expressed almost exclusively in astrocytes, where it is induced by neural injury and released upon disintegration of the astrocyte cytoskeleton (Rempe and Nedergaard, 2010). GFAP plays an essential role in maintaining shape and motility of astrocytic processes and contribute to white matter architecture, myelination and blood brain barrier (BBB) integrity (O'Callaghan et al., 2015). After traumatic brain injury (TBI), GFAP's serum concentration peaks at 2–6 h and has a half-life of <2 days (Diaz-Arrastia et al., 2014). S-100B exerts both detrimental and neurotrophic effects, depending on its concentration in brain tissues (Adami et al., 2001). After release, S-100B acts as a trophic factor for serotoninergic neurons, and plays a role in axonal growth and synaptogenesis during development. Thus, traumatic acute injury results in great destruction of astrocytes leading to massive release (50 to 100 fold) of S-100B into plasma, whereas S-100B levels in psychiatric disorders were

only about 3 times higher in patients compared to controls (Uda et al., 1998; Arolt et al., 2003), correlating well with its neuroprotective action. Specifically, S-100B stabilizes tau and MAP-2. Its half-life in the serum is 2 h (Zurek and Fedora, 2012).

A recent study of airline pilots and other flight crew members chronically exposed to organophosphates through combustion of engine oil and hydraulic fluid that contain organophosphate esters resulted in symptoms similar to those reported by GW veterans (fatigue, headaches, confusion and memory problems). Interestingly, these crew members showed significantly elevated numbers of autoantibodies in their blood serum of CNS damage markers including those associated with axonal transport (microtubule associated protein-2 (MAP-2), tubulin, neurofilament triplet proteins (NFP) and microtubule associated protein-tau (tau protein)) and those exclusively associated with CNS glial activation and neuroinflammation (myelin basic protein (MBP), and glial fibrillary acidic protein (GFAP) (Abou-Donia et al., 2013). A follow-up histopathology autopsy study was performed on a deceased pilot with organophosphate exposure that confirmed CNS damage and demyelination (Abou-Donia et al., 2014). Specifically, the histopathology results showed axonal degeneration and demyelination and the post-mortem and pathological examination of the nervous system confirmed the autoantibody biomarker results.

Recent studies with GW veterans have shown persistent signs and symptoms characteristic of CNS injury including brain imaging and cognitive studies (White et al., 2016; Chao et al., 2010, 2011, 2014, 2016; Heaton et al., 2007; Sullivan et al., 2003). There are, however, no validated objective diagnostic tests to identify acute or chronic sequelae of brain injury in this veteran group. Diagnosis of brain injury using cranial computed tomography (CT) scan and magnetic resonance imaging (MRI) techniques such as diffusion tensor imaging (DTI), have not been able to clinically diagnose veterans with GWI because there have been no proven cutoff values for volumetric or other imaging parameters that have been able to provide the required near 100% accuracy in terms of sensitivity/specificity at the individual level to distinguish cases from controls needed for a diagnostic test. Imaging studies have been able to show differences and altered CNS functioning between veterans with GWI and healthy controls but have not yet been able to identify the groups diagnostically because of the significant overlap between the groups (Chao et al., 2010, 2011, 2014, 2016; Heaton et al., 2007). Hence, it is important to develop clinically available, simple and inexpensive biomarkers for detection of neuronal and glial injury essential in the diagnosis and understanding of the temporal progression of CNS damage in GWI. Recently, serum biomarkers such as cytoskeletal proteins, resulting from axonal degeneration, have been used in diagnosing brain injury (particularly traumatic brain injury). The use of these biomarkers is usually measured in serum shortly after brain injury, because they have short half-lives (Zurek and Fedora, 2011; Diaz-Arrastia et al., 2014).

However, many years have elapsed since the time that GW veterans returned from deployment and became ill therefore, this particular approach cannot apply to GWI. Based on results from both chronic and acute injury, we used our novel autoantibody biomarker panel described above for brain injury to test for the indication of CNS damage in veterans with chronic GWI (Abou-Donia et al., 2013, 2014). One prior study compared autoantibodies of myelin basic protein (MBP) and striated muscle antibodies in GW veterans and reported higher MBP and muscle antibodies in veterans with GWI (Vojdani and Thrasher, 2004). Autoantibodies have previously been recognized as potential objective biomarkers of GWI (Golomb, 2012). Therefore, we hypothesized that chemical exposure to pesticides, anti-nerve gas pills and/or sarin nerve gas during deployment in veterans with GWI caused an excitotoxic cascade (through potential glutamatergic, oxidative stress and proinflammatory cytokine signaling) resulting in neurodegeneration and apoptotic loss of brain cells, leading to blood brain barrier leakage of specific neuronal and glial proteins into circulation, with subsequent formation of autoantibodies (AB) against these

proteins (Abou-Donia et al., 2013; Banks and Lein, 2012; Golomb, 2008; Terry, 2012; Binukumar and Gill, 2010; Soltaninejad and Abdollahi, 2009). In this study, we determined circulating IgG- class autoantibodies in serum from 20 GWI cases and 10 symptomatic (low back pain) controls against the following 9 brain proteins: neurofilament triplet proteins (NFP), tubulin, microtubule associated protein-tau (tau proteins), microtubule associated protein-2 (MAP-2), calcium/calmodulin Kinase II (CaMKII), myelin basic protein (MBP), myelin associated glycoprotein (MAG), glial fibrillary acidic protein (GFAP) and S-100B.

2. Materials and methods

2.1. Materials

The sources of proteins were: NFP (bovine spinal cord), tau protein (human), MAP-2 (bovine serum), tubulin (bovine brain), and MBP (human brain), from Sigma-Aldrich (Saint Louis, Missouri); CaMKII (Human) recombinant Protein and MAG recombinant Protein from Novus Biologicals, Littleton, CO, GFAP (human) from Biotrend Chemikalien GmbH, (Cologne, Germany) and S-100B (human brain) from American Qualex International, Inc. (San Clemente, California). Horseradish peroxidase-conjugated goat anti-human IgG, and enhanced chemiluminescence reagent were obtained from Amersham Pharmacia Biotech (Piscataway, New Jersey). SDS gels, 2–20% gradient (8 × 8), and tris-glycine 15 mM were obtained from Invitrogen (Carlsbad, California). All other materials were purchased from Amersham.

2.2. Ethics statement

Approval for the use of stored blood samples for this study was obtained from the Duke University Medical Center Institutional Review Board.

2.3. Case and control samples

Serum samples from 20 GWI cases with GWI and 10 non-veteran symptomatic controls with lower back pain were tested in this pilot study. GW veteran serum samples were collected from a study of acupuncture treatment in veterans with GWI from 2010 to 2012 (Conboy et al., 2012). Control serum samples were derived from a separate study of non-veteran patients with chronic lower back pain who served as 'symptomatic low back pain' controls from 2011 to 2013 (Jacobson et al., 2015). Veterans with GWI will be referred to as 'cases' and low-back pain symptomatic controls will be referred to as 'controls'.

2.4. Description of the patient cohorts

2.4.1. GWI-case cohort
"The Effectiveness of Acupuncture in the Treatment of Gulf War Illness" PI: Conboy, (8/21/2010–12/26/2012) N = 104; Study Site: New England School of Acupuncture (NESA). Cases were recruited through the Defense Manpower Data Base (DMDC) personnel listings and advertisements. Cases were screened for GWI symptoms and were required to meet the CDC diagnostic criteria for chronic multi-symptom illness (CMI) in order for inclusion in the parent study and in the current study (Conboy et al., 2012; Fukuda et al., 1998). Inclusion in the current study also required that veterans were deployed to the 1990–1991 Gulf War. CMI is characterized by one or more symptoms of at least 6 months duration from at least two of three symptom categories: 1) fatigue; 2) mood-cognition; 3) musculoskeletal pain.

Symptoms were not necessarily required to have started during or after the Gulf War deployment. Exclusionary criteria included that the veteran was 1) currently enrolled in another clinical trial 2) Had another disease that likely could account for the symptoms, as determined by the Medical Monitor 3) Severe psychiatric illness (in the last 2 years psychiatric hospitalization, suicidal attempt, alcohol or substance abuse, use of antipsychotic medication) 4) Unable to complete the protocol based on the evaluation of the Medical Monitor.

2.4.2. cLBP-cohort
"Structural Integration for chronic low back pain" PI: Jacobson (3/4/ 2011–6/21/2013) N = 46. Study Site: Spaulding Rehabilitation Hospital (SRH). In this cohort, 46 outpatients from the Boston area with chronic nonspecific low back pain were randomized to parallel 20-week long treatment groups of structural integration (SI) plus outpatient rehabilitation (OR) versus OR alone. The details of the study are described in a recent publication (Jacobson et al., 2015). Inclusion criteria for the parent study included: (i) Men and women aged 18–65, (ii) cLBP of ≥6 months duration, not attributed to infection, neoplasm, severe radiculopathy (as indicated by frequent severe pain radiating down a leg), fracture, or inflammatory rheumatic process, (iii) bothersomeness of back pain self-rated on average over the preceding 6 months ≥3 on an 11-point ordinal scale (0 = none, 10 = worst imaginable), (iv) prior arrangement to enter a course of outpatient physical therapy for low back pain at a Boston area rehabilitation clinic, (v) English language fluency and mental capacity sufficient to provide informed consent and participate in the study. Exclusion Criteria for the study included: (i) Impaired hearing, speech, vision, and mobility sufficient to interfere with participation in the study, (ii) current or anticipated receipt of payments from Worker's Compensation or other insurance for disability attributed to low back pain, (iii) prior treatment with any SI therapy, (iv) plans to initiate additional treatment for back pain during the period of the study other than outpatient rehabilitation care, particularly massage or other manual therapies (e.g., chiropractic or osteopathic manipulation), (v) exclusions for safety: unresolved musculoskeletal pathology of the lower limbs, current pregnancy, any implanted medical device, osteoporosis, any hypercoagulation condition, eczema, skin infection, deep vein thrombosis, burns or other acute trauma including unhealed bone fractures or open wounds, psoriasis, psychiatric illness not well controlled, or current episode of exacerbated major depressive disorder.

2.5. Collection and storage of samples

Samples from the GWI-cohort and the cLBP-cohort were all collected from the Boston area at the same time period at two different sites from 2010 to 2013. All sites followed exactly the same protocol for venipuncture, blood handling, serum separation, aliquoting and storage at −80 °C. The same phlebotomy and sample protocol was distributed in writing to all sites. All samples analyzed were baseline blood samples collected pre-intervention therapy. Samples used for this study have not been previously thawed and are free of hemolysis by visual inspection (Tuck et al., 2009).

2.6. Participant demographics

The participant demographics indicate that a total of 20 veterans with GWI, 18 males and 2 females, compared to 6 females out of 10 cLBP controls participated in the study. The age of the GWI cases ranged from 38 to 61 (mean ± SD 46.0 ± 6.8) compared to 25 to 64 (mean ± SD 50 ± 11.4) years for controls; all study participants were white (Table 1). Seventy percent of veterans with GWI reported taking PB

Table 1
Study participant demographics.[a]

Demographics	Cases	Controls
Age (mean ± SD)	46 (6.4)	50 (11.4)
Gender (% female)[*]	10	60
Race (% Caucasian)	100	100

Age range of Cases = 38–61 years and Controls = 25–64 years in 2010–2013 when the blood was collected.

[a] A total of 20 cases and 10 controls participated in the study.

[*] Cases were significantly different from controls for gender p < 0.05 but not for age.

pills during the war (n = 14). The groups differed with respect to gender (X2 = 8.5; p < 0.05) with significantly more women in the control group but did not differ with respect to age (t-value = − 1.3; p > 0.05).

2.7. Western blot assay

To screen for the presence of autoantibodies against a battery of proteins, we applied a Western blot approach as previously reported (Abou-Donia et al., 2013). Each serum sample was analyzed in triplicate. Each protein was loaded as 10 ng/lane except for IgG that was loaded as100 ng/lane. Proteins were denatured and electrophoresed in SDS-PAGE (4% to 20% gradient) purchased from Invitrogen (Carlsbad, CA). One gel was used for each serum sample. The proteins were transferred into polyvinylidene fluoride (PVDF) membranes (Amersham Pharmacia Biotech Piscataway, New Jersey). Nonspecific binding sites were blocked with Tris-buffered Saline-Tween (TBST) (40 mM Tris [pH 7.6], 300 mM NaCl, and 0.1% Tween 20) containing 5% non-fat dry milk for 1 h at 22 °C. Membranes were incubated with serum samples at 1:100 dilutions in TBST with 3% non-fat dry milk overnight at 4 °C. After five washes in TBST, the membranes were incubated in a 1:2000 dilution of horseradish peroxidase-conjugated goat anti-human IgG (Amersham Pharmacia Biotech (Piscataway, New Jersey). The dot blots were probed with anti-human IgG (H + L) HRP conjugate antibody (Cat. No. 31410, Thermo Fisher Scientific Inc., Pittsburgh, PA, USA) for 1 h at RT, incubated with ECL reagent (Cat. No. 34096). The membranes were developed by enhanced chemiluminescence using the manufacturer's (Amersham Pharmacia Biotech) protocol and a Typhoon 8600 variable mode imager. The signal intensity was quantified using Bio-Rad image analysis software (Hercules, California). All tests were performed with the investigators blinded to participant diagnosis.

2.8. Specificity of serum autoantibodies

Previously we checked the specificity of the serum autoantibody by performing peptide/antigen competition assay, in which the serum was spiked with the target protein or peptide (Abou-Donia et al., 2013). The serum from random healthy controls was mixed with or without tau, MAP or MBP. The serum/protein mix was centrifuged at 15,000 rpm to pellet any immune complexes. The supernatants were then carefully removed and used in Western blotting.

2.9. Calculations

The mean value of the optical density measurement from the triplicate testing was used for each serum sample tested and normalized by total IgG. Thus, the results are expressed as mean values of triplicate assays of optical density arbitrary units normalized to total serum IgG.

2.10. Power analysis

A total of 20 GWI cases were available for testing in this convenience sample. Effect size calculations were based on two-sample t-test assuming a common standard deviation between groups. The power analysis assumes that cases and controls are not matched. In a t-test of difference between two independent means, selecting power of 80%, 2-sided alpha 0.05, and size of 20 vs 10, the study was powered to detect an effect only if at least 1.12 SD.

2.11. Statistics

Grouped data are reported as mean ± SD. The values from cases were compared to the control group using t-tests and Pearson correlation analyses (SigmaStat, Systat Software) and p-values were
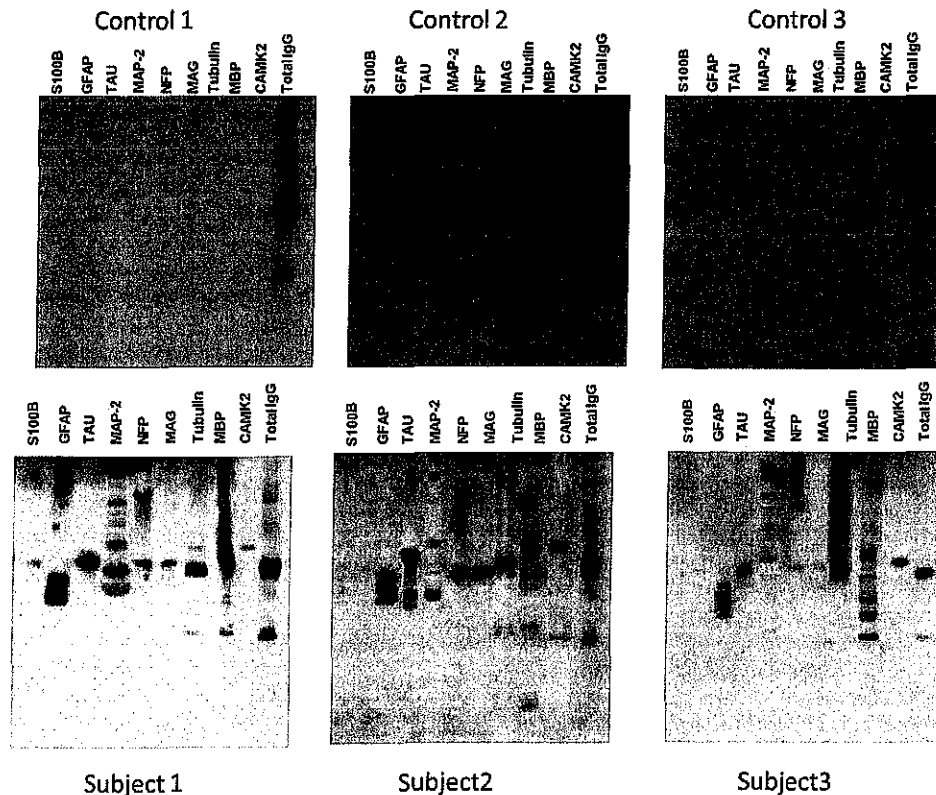
Fig. 1. A representative sample of Western blot gels from three cases showing that the majority of GWI serum reacted intensely to neural proteins, while most control serum showed a weak or no reaction.

Table 2
Chemicals, environmental and other exposures of cases during the Gulf War.[a]

Chemical exposures	Exposed	%	Environmental and other exposures	Exposed	%
Pyridostigmine bromide (PB)	14	70	Khamisiyah notification letter	8	40
Organophosphorus pesticides (OP)	7	35	Contaminated food/water	18	90
Carbamates	7	35	Vaccines	18	90
Pyrethroids	4	20	Malaria	12	60
DEET	11	55	Sand	18	90
Sarin	9	45	Tent heater	11	55
Depleted uranium (DU)	6	30	Jet fuel	14	70
Solvents	10	50	Oil fires	18	90

[a] A total of 20 veterans with GWI participated in the study.

calculated. Pairwise correlations among the nine biomarkers were assessed. A 2-sided p value <0.05 was considered significant. Due to the exploratory nature of this pilot study, analyses were not adjusted for multiple comparisons.

3. Results

As previously described, we assessed the specificity of the serum autoantibody by performing peptide/antigen competition assay, in which the serum was spiked with the target protein or peptide. The serum bound to tau eliminated the tau band in the Western blot (see Fig. 1) while the band of MAP-2 or MBP were present and not affected. The serum bound to MAP-2 eliminated the MAP-2 band in the Western blot while the band of tau or MBP was present. The serum bound to MBP eliminated the MBP band in the Western blot while the bands of tau and MAP-2 were present. These results indicate that each autoantibody in the serum was specifically neutralized by its target protein in serum sample and was no longer available to bind to the epitope present in the protein on the Western blot. This confirmed that the assay used in this study, was specific and accurately determined autoantibodies against tested proteins in serum samples.

To detect autoantibodies in serum, we probed Western blots with individual serum samples. A total of 30 human serum samples (20 veterans with GWI and 10 non-veteran symptomatic low-back-pain controls) underwent measurement of the levels of the serum circulating IgG-class autoantibodies against nine neuronal- and glial- specific proteins. Table 2 lists the number of GWI cases who were exposed to chemical and environmental exposures. It shows that 14 cases (70%) used PB as a prophylaxis against possible exposure to nerve agents and nine cases reported being exposed to the nerve agent sarin. In addition, a total of eight cases reported receiving notification from the Department of Defense (DOD) that they were potentially exposed to sarin and other chemicals due to their proximity to the Khamisiyah, Iraq underground weapons depot where a chemical weapons cache was destroyed in March 1991 (US DOD, 2002). Eight cases reported exposure to depleted uranium. All of the cases reported exposure to one or more insecticides or a mixture of pesticides including organophosphates, carbamates,

pyrethroids and organochlorines. Eleven cases used the insect repellant DEET. All cases underwent environmental and other exposures listed in Table 2. Other chemicals that the cases reported exposure to included oil well fires, sand, tent heaters, jet fuel, and solvents. Some veterans reported exposure to malaria and 18 reported being vaccinated. Serum from GWI cases showed significantly increased levels of autoantibodies against all cytoskeletal proteins except those against S-100B compared to non-veteran symptomatic (low back pain) controls (Table 3). Due to the gender differences between the cases and controls, analyses were also run with just the males in the groups. Although there was only a small number of males (n = 4) in the control group which could be problematic in this type of analysis, results of this comparison showed a very similar pattern of significant differences in all autoantibodies (GFAP p < 0.001; Tau p < 0.001; MAP p < 0.002; MAG p < 0.001; PNF p < 0.006; Tubulin p < 0.003; MBP p < 0.01; S-100B p = 0.31). The majority of GWI serum reacted intensely to neural proteins, while most control serum showed a weak or no reaction. Fig. 1a and b present Western blots results from three representative GWI cases and three controls. The levels of serum autoantibodies in GWI cases and controls to neural-specific proteins expressed as mean values ± SD of triplicate assays of optical density arbitrary units normalized to total serum IgG optical density ranged from 0.30 for S-100B and 4.09 for GFAP for the cases compared to 0.30 and 0.62, respectively for controls are listed in Table 3 and shown in Fig. 2. The percentage of autoantibodies against neural proteins of cases compared to controls (in descending order) were: CaMKII, 927, GFAP 660, Tau 483,Tubulin 441, MAG 360, MBP 250, NFP 245, MAP-2 230, S-100B 103. Fig. 3 presents the mean values ± SD (p < 0.001) of fold increase of autoantibodies against neural proteins for the cases compared with the controls. Serum from controls had no or low levels of circulating autoantibodies to nervous system-specific biomarkers. Autoantibodies against CaMKII were more predominant in the cases' serum than in controls' serum (Fig. 3).

Fig. 4 shows that Tubulin and GFAP had the highest values in the GWI cases compared with the controls. Pairwise correlations among the nine autoimmune biomarkers were significant only for the pair Tau and MBP. When comparing the correlation between each pair, only tau and MBP were significantly linearly correlated to each other (Fig. 5). Fig. 5 shows that the control values of those two biomarkers were <1 optical density unit, whereas GWI cases had values strongly linearly correlated with each other such that on average tau was elevated up to 10 times higher than controls in some GWI cases, and MBP was also elevated up to 5 times higher for the same cases vs the controls.

Finally, when each biomarker was compared separately between individual cases and controls for potential fold-increase cut-points to discriminate the groups, results indicated that tubulin values had some of the highest-fold increased values in the individual GWI cases compared with the individual control values although only 60% of the individual cases (n = 12) showed that effect (Fig. 6a). However, in 9 (out of the 20) cases tubulin values were elevated by a factor of 3 to 9-fold higher than the controls. In Fig. 6b, GFAP was elevated the most in cases compared to controls. In fact, GFAP was higher in all of the cases compared with all of the controls with 20 out of 20 cases having 2 to 7 fold higher

Table 3
Statistical analysis of the levels[a] of serum autoantibodies (AA) in controls[b] and GWI cases[b] to neural-specific proteins.

	NFP	Tau	Tubulin	MBP	MAG	MAP2	GFAP	S-100B	CaMKII
Cases Mean ± SD	1.42±0.24	2.52±0.31	3.48±0.78	1.75±0.30	1.44±0.28	2.18±0.29	4.09±0.33	0.30±0.03	1.02±0.20
Controls Mean ± SD	0.58±0.09	0.60±0.09	0.79±0.11	0.70±0.11	0.40±0.04	0.086±0.09	0.62±0.11	0.29±0.04	0.11±0.03
p values	0.02	0.0001	0.001	0.001	0.007	0.002	0.00001	0.40	0.015

[a] The results are expressed as mean values of ± triplicate assays of optical density arbitrary units normalized to IgG optical density as fold of healthy controls.
[b] Values from cases were compared to the control group using t-tests; most were highly significant p < 0.001 (2-sided), except for S-100B that was not significantly different from controls. Cases were significantly different from controls with respect to gender p < 0.05 but not with respect to age.
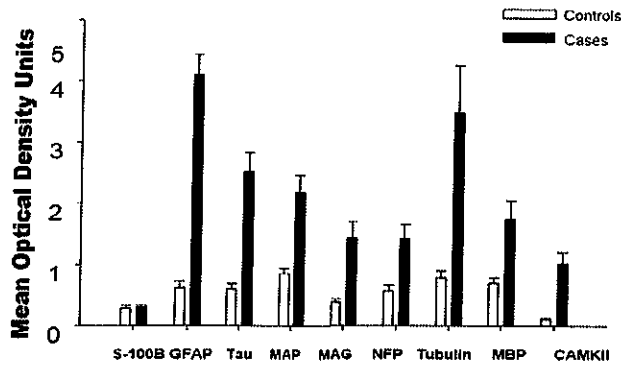
Fig. 2. Mean autoantibodies against neural proteins from cases and controls expressed in mean optical density units.

value than the control mean. Thus GFAP values completely distinguished the cases from the controls. GFAP values did not overlap in cases vs controls in this small sample; however, the separation in the ranges was small relative to the substantial standard deviations. In Fig. 6c, tau was higher than controls in 18 cases and 50% of the cases had double the value of tau compared with the controls. In Fig. 6d, MAP was higher than the controls in 15 cases and 75% of the cases had a 0.5 to 11-fold higher value than the controls. In Fig. 6e MAG was higher than controls in 15 cases and 75% of the cases had up to a 10-fold higher value than the controls. In Fig. 6f NFP was higher than controls in only 50% of the cases (n = 10) and they showed 0.5 to 11-fold higher values than controls. MBP was higher than controls in 12 cases and 60% of the cases were higher than controls with 2 to 5-fold higher values than controls. CAMKII was higher than controls in 16 cases and 50% of the cases had a 3 to 30-fold higher value than the controls. S100B values were not statistically significant as the values overlapped with cases and controls.

4. Discussion

This pilot study reports significantly elevated levels of autoantibodies against neurotypic- and gliotypic-specific proteins in serum from a sample of 20 veterans with GWI and 10 non-veteran symptomatic (low back pain) controls with musculoskeletal symptoms rather than CNS symptoms. The increased levels in GWI cases compared to controls ranged from 9.27 fold for CaMKII to 6.6 fold for GFAP to 2.45 fold for neurofilaments. Autoantibody levels against S-100B were not different in GWI cases than controls (1.03 fold) consistent with its neural protective role and in agreement with presence of chronic injury and
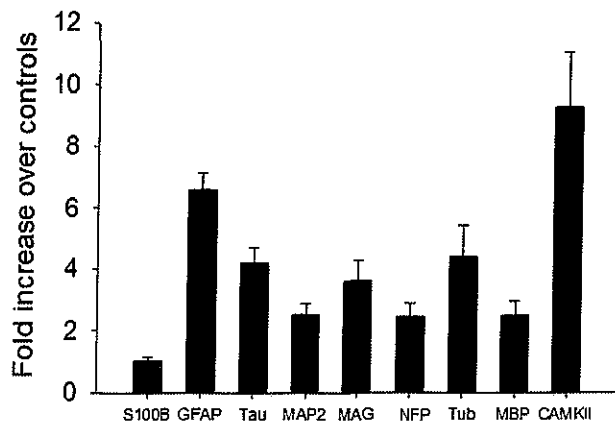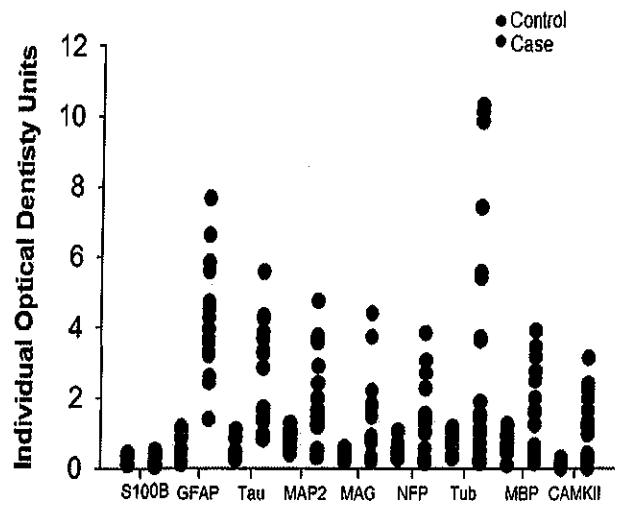

Fig. 4. The levels of autoantibodies of neural proteins of GW cases and of controls expressed as optical density units.

absence of acute brain injury in veterans with GWI (Zurek and Fedora, 2011; Diaz-Arrastia et al., 2014; Stalnacke et al., 2006, 2004; Coch and Leube, 2016). Previous studies, using animal models of GWI, showed that exposure to the neurotoxicants that were present in the GW environment, caused deficits in behavioral outcomes that were accompanied by neuronal and glial degeneration (Abdel-Rahman et al., 2001, 2002a,b, 2004a,b; Abou-Donia et al., 2000, 2001, 2004). Following neurodegeneration, there is accumulation of cellular neurological waste products or debris such as misfolded or hyper-phosphorylated proteins that form toxic stable aggregates (Nedergaard, 2013; Edgar et al., 2004). This extracellular debris send damage signals that cause the CNS immune cells - microglia to become activated and act as profound antigen presenting cells that secrete pro-inflammatory cytokines (IL-1b, TNF-a and IL-6) and mediators (reactive oxygen species, ROS) resulting in the recruitment of T-lymphocytes (Milligan and Watkins, 2009; Banks and Lein, 2012). Multiple exposures to these waste proteins can cause microglia and astrocytes to become primed to react more strongly after each subsequent exposure (Watkins and Maier, 2003). This can result in a persistent neuroimmune response and chronic neuroinflammation contributing to chronic health symptoms, such as those seen in GW veterans (Johnson et al., 2016; Milligan and Watkins, 2009; Maier and Watkins, 1998; Watkins and Maier, 2003). These waste proteins are eventually released into circulation due to defects in the
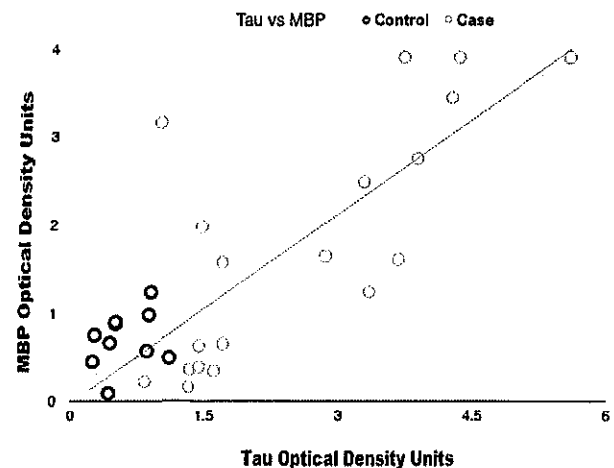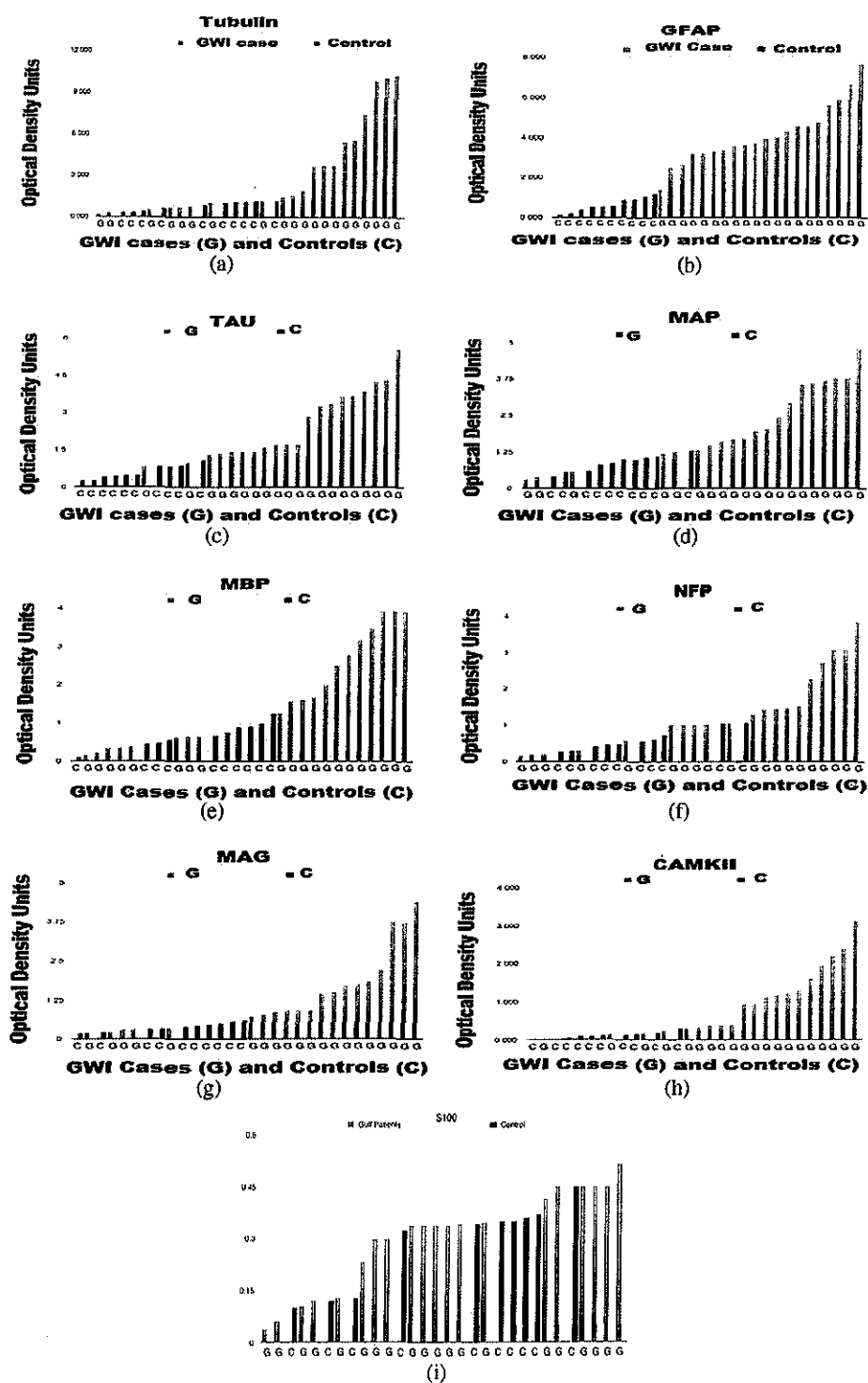

Fig. 3. Folds increase of autoantibodies against neural proteins from cases relative to controls.

Fig. 5. Paired correlations of Tau and MBP optical density levels in cases relative to controls.

Fig. 6. a) Tubulin levels were higher than all controls in 12/20 cases. b) GFAP levels were higher than all controls in 20/20 cases. c) Tau levels were higher than all controls in 17/20 cases. d) MAP levels were higher than all controls in 15/20 cases. e) MBP levels were higher than all controls in 12/20 cases. f) NFP levels were higher than all controls in 10/20 cases. g) MAG levels were higher than all controls in 15/20 cases. h) CAMKII levels were higher than all controls in 16/20 cases. i) S100B levels overlap with cases and controls.

brain-blood barrier induced by astrocyte alterations. Waste proteins in the brain ultimately reach the liver through a mechanism known as the "glymphatic system" where they are degraded (Nedergaard, 2013). However, the released proteins that could serve as markers of injury are present in the short-term and cannot be used as biomarkers in the case of chronic GWI (Zurek and Fedora, 2011; Diaz-Arrastia et al.,

2014). Thus detection of autoantibodies can serve as surrogate markers for these circulating waste proteins as described in this study.

The highest increase in autoantibodies was against CaMKII which was 9.27 times higher than that of controls followed by GFAP which was 6 times higher than controls. This result is consistent with the veterans' exposure during their deployment to the Gulf War to

organophosphorus compounds such as pesticides, and the nerve agent sarin that have been shown to increase the activity and mRNA expression of CaMKII (Patton et al., 1983, 1985, 1986; Gupta et al., 1998; Barbier et al., 2009) as well as enhanced CaMKII-induced phosphorylation of NFP, tubulin (Serrano et al., 1986) and tau activity leading to the aggregation, deregulation and accumulation of NFP (Abou-Donia et al., 1993; Norgren et al., 2003) and tubulin in the axon (Abou-Donia, 1993; Jensen et al., 1992, Gupta et al., 2000; Grigoryan and Lockridge, 2009). Aggregated neurofilaments result in slowing of axonal transport as has been illustrated in GW-relevant animal and cell neurotoxicant models (Gupta et al., 1997; Reagan et al., 1994; Terry et al., 2012; Gao et al., 2016; Edgar et al., 2004). GW-relevant exposure models have also been associated with astrocyte activation (Zakirova et al., 2015; Ojo et al., 2014).

Neuronal proteins studied in this pilot analysis represented various anatomical regions of the neuron with distinct functions which can be instructive with regard to the pathobiology of GWI (Lapadula and Abou-Donia, 1992). All of the proteins used are involved in axonal structure and function and are released as products of neural degeneration of various regions of the neuron. MAP-2 is present in the dendrites; CaMKII, tau, tubulin, and neurofilament proteins are located in the axon; myelin basic protein (MBP) and myelin associated glycoprotein (MAG) are an integral part of myelin (McMurray, 2000). Furthermore, the central nervous system-specific glial protein, GFAP and S-100B are secreted by astrocytes after neuronal injury (McMurray, 2000). Following axonal and myelin degeneration, neuronal and glial proteins are released and once in circulation, activated lymphocytes, B and T cells lead to the formation of autoantibodies against these proteins (Schwartz and Shechter, 2010a,b).

Increased autoantibodies against nervous system-specific proteins leads to structural consequences in various regions as follows: increased autoantibodies against neurofilaments proteins, tau, CaMKII and tubulin are indicative of axonal degeneration; increased autoantibodies against MAG and/or MBP suggest demyelination, increased autoantibodies against MAP-2 suggest dendritic degeneration, increased autoantibodies against GFAP suggest astrogliosis, and the low or no-increased levels of autoantibodies against S-100B is consistent with chemical-induced brain injury (Zurek and Fedora, 2011, Diaz-Arrastia et al., 2014; Stalnacke et al., 2006, 2004). The linear correlation pattern of tau and MBP in this study suggests an important potential effect of axonal degeneration followed by demyelination that would correspond with prior neuroimaging studies in neurotoxicant exposed GW veterans (Heaton et al., 2007; Chao et al., 2010).Furthermore, these structural changes of the nervous system lead to functional alterations. Hence axonal degeneration in the cerebral cortex leads to: motor and sensory abnormalities, ataxia, deficit in posture, locomotion, and skilled fine motor movements (fingers, speech, facial expression) and weakness; degeneration of the limbic system including the hippocampus leads to: learning and memory deficits, and neurobehavioral (mood, emotion and judgment) abnormalities; increased autoantibodies against MAP-2 suggests damage to the dendrite-rich Purkinje cells in the cerebellum resulting in: gait and coordination abnormalities, staggering gate and ataxia (McMurray, 2000; Abou-Donia, 2015). Increased autoantibodies against GFAP indicate astrogliosis and potential neuroinflammation and/or glial scarring. GFAP contributes to white matter architecture, myelination and blood brain barrier (BBB) integrity (O'Callaghan and Sriram, 2005; Amourette et al., 2009; Lamproglou et al., 2009). Consequently, blood levels of GFAP in healthy individuals are very low. GFAP levels were higher in GWI cases and completely discriminated between the cases and controls in this study. This is particularly relevant because disorders with higher levels of GFAP include memory disorders such as Alzheimer's and vascular dementia that have significant axonal neurodegeneration and neuroinflammation (Mecocci et al., 1995). Increased autoantibodies against S-100B suggest traumatic brain damage and can help to differentiate between acute and chronic brain injury (Stroick et al., 2006; Stalnacke et al., 2006, 2004; Zurek and Fedora,

2011; Diaz-Arrastia et al., 2014; Coch and Leube, 2016). Their lack of increase in this study suggests against acute traumatic brain injury in veterans with GWI.

Important mechanistic clues from animal and cell studies of these GW-relevant neurotoxicants have shown deficits in axonal transport, as well as aberrations in neurofilaments and microtubules, which are the structural railways for axonal transport (Gupta and Abou-Donia, 1995a, b; Gearhart et al., 2007; Grigoryan and Lockridge, 2009; Prendergast et al., 2007, Jiang et al., 2010). Mitochondria are also delivered by axonal transport to provide the energy required to power the biochemical reactions necessary for the functioning of the axon and have shown altered functioning in GW-relevant neurotoxicant models (Middlemore-Risher et al., 2011). GW-relevant chronic low-level organophosphate exposure has also been associated with mitochondrial compromise from oxidative stress induction and with neuroinflammation resulting in cell damage or cell death resulting in debris of waste proteins in the extracellular spaces (Laetz et al., 2009; Kaur et al., 2007; Banks and Lein, 2012). In fact, one hypothesis of GWI suggests that mitochondrial damage and oxidative stress in the brain and the periphery have caused the chronic symptoms of GWI; notably, increased autoantibodies were expressly cited among objective markers and mediators in this model (Golomb et al., 2014; Golomb, 2012; Koslik et al., 2014).

Another hypothesis of GWI suggests that the neurotoxicants acted synergistically to create a self-perpetuating neuroinflammatory state, which in turn has an ongoing negative impact on brain cells including neurons (microtubules, motor proteins, mitochondria) and glia (microglia, astrocytes, oligodendrocytes) and blood-brain barrier function (O'Callaghan and Sriram, 2005). Clinical studies have also found consistent results with GW veteran cohorts who showed impaired cognitive functioning and reduced volume and altered white matter microstructural integrity on MRI in OP pesticide, sarin nerve agent and PB pill exposed cohorts (White et al., 2016; Sullivan et al., 2013; Chao et al., 2010; Heaton et al., 2007; Proctor et al., 2006; Sullivan et al., 2003). These prior results suggest clear CNS alterations in neurotoxicant-exposed GW veterans which correlated with behavioral outcomes that are related to neurodegeneration and perhaps with both a chronic neuroinflammatory and Mitochondrial/OS hypothesis.

The only other study that we are aware of that compared CNS autoantibodies in GW veterans compared MBP and striated and smooth muscle antibodies and reported higher MBP and muscle antibodies in veterans with GWI when compared with controls (Vojdani and Thrasher, 2004). The current study validates the prior MBP findings and expands on those findings with a larger panel of 8 additional CNS autoantibody markers. Collectively, these findings suggest that alterations in white matter as evidenced by circulating autoantibodies to MBP appear to be associated with GWI. This finding corresponds with both leading hypotheses for GWI given that white matter alterations can be associated with oxidative stress and neuroinflammation as a result of glial activation and signaling of both proinflammatory cytokines and oxidative stress (Milligan and Watkins, 2009). The additional finding of this study that higher Tau autoantibody levels were significantly linearly correlated with higher MBP autoantibody levels in GWI cases suggests that axonal degeneration may be occurring before demyelination in veterans with GWI and warrants further more conclusive study to distinguish it from the more myelin-specific toxic leukoencephalopathies (Schmahmann et al., 2008; Filley, 2013). These findings also correspond with MRI findings of differences on both white and gray matter brain volumes in neurotoxicant-exposed GW veterans (Heaton et al., 2007; Chao et al., 2010, 2011, 2014, 2016). These findings also clearly suggest that glia and astrocytes in particular should be further studied in GWI given significantly higher levels of GFAP in the GWI cases that correspond with prior animal models of GWI (Abdel-Rahman et al., 2001, 2002a, 2002b, 2004a, 2004b; Abou-Donia et al., 2000, 2001, 2002, 2004; Zakirova et al., 2015; Ojo et al., 2014) and with recent studies illustrating the ability of astrocytes to donate mitochondria to damaged neurons (Hayakawa et al., 2016).

4.1. Limitations and future directions

This study, like all studies has important limitations. Although the present pilot study can serve as a proof-of-concept it has a small sample size and non-matched subject groups for age, gender and for CNS symptoms. This is particularly important as it has also been shown that CNS autoantibodies have been reported to be age-related in animal models (Lal and Forster, 1988). In addition, the convenience comparison group utilized in this study had musculoskeletal symptoms and not CNS symptoms therefore, it remains to be shown that these CNS autoantibody markers can clearly distinguish between GWI cases and additional groups with CNS specific symptoms. However, the strong results including 9-fold higher levels of CAMKII, 6-fold higher levels of GFAP and 4-fold higher levels of tau and tubulin that were presented in this study warrant further research for a blood-based objective marker of GWI in larger, well-characterized veteran cohorts. These results suggest a possible new avenue for further development of an objective biomarker of GWI. The identification of this small panel of neural-specific autoantibody biomarkers in GWI shows promise for further validation in larger study samples that are more carefully matched for subject demographics (particularly age), different types of control groups (i.e. healthy and CNS symptomatic groups) and that classify cases by both the CDC and the more specific Kansas GWI criteria which also specifies the time period of deployment which may be relevant to particular OP and other deployment-related exposures (Steele, 2000; Fukuda et al., 1998). Future directions will be to compare these CNS autoantibody markers with specific behavioral outcomes including cognitive performance and brain imaging of gray and white matter volume and microstructural integrity to further validate these suspected brain-immune-behavioral outcomes.

5. Conclusions

In conclusion, in this pilot study GWI was significantly associated with 2–9 fold increased serum autoantibodies against 8 neuronal- and glial-specific proteins (CaMKII, GFAP, Tau, Tubulin, MAG, MBP, NFP, MAP-2) and not with a marker of more acute damage (S-100B). The autoantibodies that were found here to be elevated in GWI, targeted proteins/ antigens that play critical roles in the structure and function of the neuron including axonal transport and myelination. Many of them are explicit markers for neurodegenerative disorders, consistent with axonal and myelin degeneration of myelinated neurons and with astrogliosis, cell signaling and neuro-inflammation. These same proteins have been shown to be affected in other clinical groups and animal models with similar organophosphate and carbamate exposures (Abou-Donia et al., 2013, 2014). These results validate prior reports of increased MBP autoantibodies in GWI cases and suggest that oligodendrocyte signaling, glia and white matter alterations should continue to be further studied in GWI and validated with health symptom and behavioral outcomes (Vojdani and Thrasher, 2004). The results also indicate that veterans with GWI may be continuing to show brain neuronal degeneration and glial activation that would be consistent with recent reports of chronically persistent and in some cases worsening health of these veterans (Smith et al., 2013; Ozakinci et al., 2006; Li et al., 2011; Kang et al., 2009; Dursa et al., 2016; White et al., 2016). These results suggest a possible avenue for further development of a panel of objective biomarkers of GWI upon further validation in larger study samples that are more carefully matched for subject demographics.

Conflict of interest statement

The authors report no relationships that could be construed as a conflict of interest.

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ACUPUNCTURE
Today

Treating Gulf War Illness: The Lasting Effects of Desert Shield/Storm

By Joe C. Chang, MAOM, Dipl. OM, LAc, Rosa N. Schnyer, DAOM, CFMP, LAc and Lisa Conboy, ScD

Clinical and registry programs indicate that 25 percent of the 700,000 veterans of the first Gulf War (Operation Desert Shield/Storm, years 1990-1991), have been affected by clusters of symptoms, and co-morbid medical diagnoses. Symptoms include chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, arthralgia, digestive complaints, and mood-related psychiatric disorders, including depression, post traumatic stress disorder (PTSD), and other anxiety disorders.[1,2]

Gulf War Illness (GWI) is a complex and difficult medical condition to treat, with highly individualistic symptom presentations, including fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain. The Centers for Disease Control and Prevention (CDC) has defined Gulf War Illness as three symptom clusters that includes: fatigability (fatigue 24 hours or more after exertion), mood and cognition (feeling depressed, irritable, anxious, difficulty in concentrating, problems getting to sleep), and musculoskeletal (joint or muscle pain).

GWI & Acupuncture

The complex diagnostic and treatment process of Chinese medicine, which is tailored to each individual's clinical presentation, can provide an effective framework for evaluating and addressing the complex constellation of symptoms presented in GWI. Currently, no biomedical standard of treatment care exists. One study, completed by the *New England School of Acupuncture,* included an unblinded phase II Randomized Controlled Trial (RCT), which offered individualized acupuncture treatments, using the available community resources.

The treatment schedule duration, dose, and specific Chinese medicine techniques employed were based on the clinical experience of the expert practitioners, and informed by literature review. Details of the protocol and implementation were determined before the trial began via focus groups with senior acupuncture faculty.

Case Study Team

Licensed acupuncturists with at least five years of clinical experience, who received additional in-house training concerning GWI, provided the acupuncture treatments. Although there are many styles of acupuncture within Chinese medicine, acupuncturists were chosen who self-reported use of the TCM model of diagnosis.

During the first session, the acupuncturists conducted an interview reviewing the subject's medical history, symptoms and aspects of diagnosis from the perspective of TCM, including condition of the tongue, pulse, meridians, and acupoints.

Each subject received an individualized diagnosis and treatment protocol addressing his or her unique pattern of symptoms. Brief interviews began each subsequent session, allowing patient and practitioner to prioritize

symptoms, and identify any questions or concerns.

Individualized treatment protocols allowed the practitioners to alter the treatment plan based on how the patient presented at the moment; including varying the selection of acupoints across treatments and adding particular co-interventions commonly used as part of TCM therapy to supplement manual needling.

For example, electroacupuncture for its efficacy in reducing pain and inflammation24, heat therapies (e.g. heat lamp), Chinese massage, and press balls, tacks or magnets applied to points after needling. Each session lasted approximately one hour. Acupoints were stimulated manually until "obtaining de qi," a technique characteristic of TCM to elicit a response felt by both the patient and the acupuncturist.

This needling sensation, adjusted for the comfort and safety of each patient, may be experienced as a pinch that rapidly subsides, or a sense of spreading pressure, dull ache, or warmth. Needles were retained for 30-45 min (10-35 stainless steel, disposable needles per session).

After needle insertion, subjects were left to rest or nap. The type of needle, including gauge (32-38) and length (15-50 mm) as well as the depth of insertion (subcutaneous to about 25 mm) varied according to the area of the body being treated (i.e. extremities vs. trunk). Choice of acupoints could vary during subsequent treatments to improve results. Herbs and supplements were not allowed. Subjects were encouraged (but not required) to remain with the same acupuncturist for the whole study period to allow for development of patient-practitioner rapport.[3]

Local advertisements and direct mailings to veterans of the first Gulf War, drawn from the Defense Manpower Data Center, recruited participants for the case study. Since the demographics of GWI veterans are unpublished, it was not known if there would be a sufficient population near the study offices to draw a fair sample. Therefore, the study was designed to include treatment sites within a 100-mile radius of the study offices, and incorporated a mechanism to add treatment sites within that radius in areas where GWI veterans were found clustered.

Thirty treatment sites were utilized. This design had the added benefit of allowing veterans to receive treatments near where they lived and worked, a technique that may have improved adherence. The resulting study provided treatments at extant acupuncturist offices to 104 veterans with GWI. The results of the study were overwhelmingly positive, achieving both clinically and statistically significant levels of improvement.

Randomized to six months of either bi-weekly acupuncture treatments or two months of waitlist followed by weekly acupuncture treatments, 82 percent of the veterans completed the protocol. Measurements were taken at baseline, two, four and six months to evaluate physical function (SF-36 physical component, SF 36P) and pain (McGill Pain scale).

Veterans who received twice per week treatments, experienced a clinically and statistically significant improvement in both pain and function at sixth month, compared to veterans receiving treatment just once per week, who also experienced a benefit in some scales such as severity of main and secondary complaint Levels of satisfaction with treatment and confidence with acupuncture and the acupuncturist were also very high, all at least 95 percent.[4]

Please note that in 2012 Congress appropriated funds to VAs for veteran treatments received in the community. For information on how to receive reimbursement for treating veterans in your clinic, please email Lisa Conboy at: **lisa_conboy@hms.harvard.edu** .

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Joe C. Chang is a second-generation acupuncturist and has worked as an acupuncturist and researcher at two integrative post-traumatic stress disorder (PTSD) programs for the United States Army. Joe C. Chang, MAOM, Dipl. OM, L.Ac., is a second-generation acupuncturist and has worked as an acupuncturist and researcher at two integrative post-traumatic stress disorder (PTSD) programs for the United States Army.

Dr. Rosa N. Schnyer is a certified functional medicine practitioner, a clinical assistant professor at the College of Nursing at the University of Texas, Austin, as well as adjunct faculty at the Oregon College of Oriental Medicine (OCOM) DAOM program, and the AOMA DAOM program. Dr. Schnyer is past co-president of the Society for Acupuncture Research.

Lisa Conboy is the director of the research department at the *New England School of Acupuncture* at MCPHS University, and an instructor in medicine, part-time at Beth Israel Deaconess Medical Center, Harvard Medical School. Lisa has a MA (Medical Sociology) MS (Public Health) ScD (Public Health).

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PLOS ONE

Characteristics of Gulf War Illness participants in an acupuncture study
--Manuscript Draft--

Corresponding Author:	Lisa Jean Taylor-Swanson, PhD, MAcOM University of Utah Salt Lake City, UT UNITED STATES

Abstract:

BACKGROUND: Gulf War veterans reported significantly more often nonspecific multiple complex medical symptoms, including fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain.
METHODS: We analyzed baseline characteristics reported by Gulf War Illness (GWI) study participants. The data was from a pragmatic randomized clinical trial to test the effects of individualized acupuncture treatments offered in extant acupuncture practices in the community. Veterans with diagnosed symptoms of GWI were included in the trial. This report focuses on sociodemographic characteristics, the SF-36 physical component scale score (SF-36P), and the McGill Pain scale at baseline.
RESULTS: Of the 192 participants assessed for eligibility, 104 subjects underwent randomization. Mean age was 48 years, 13% were female and over 2/3 self-described white in both groups. Over 2/3 reported diseases of the musculoskeletal system and connective tissue and over half reported symptoms, signs and abnormal clinical or laboratory findings. Over 1/3 of participants indicated that they were currently diagnosed with anxiety (36% of the sample), depression (35%) and Post Traumatic Stress Disorder (33%). Other concurrent diagnoses included sleep apnea (28%) and Chronic Fatigue Syndrome (28%).
DISCUSSION: Gulf War Veterans in the present study diagnosed with Gulf War Illness report many similar symptoms and diagnoses to GW veterans studied elsewhere. Specifically, increased mental health conditions such as depression, anxiety, and PTSD were identified in this sample, as well as physical conditions of Chronic Fatigue Syndrome and unidentified symptoms. Safe and effective interventions for these symptoms and condition need to be identified and studied for this population.
CONCLUSIONS: Further research is needed to identify and test safe and effective interventions for symptoms and conditions experienced by Gulf War veterans.

Order of Authors:	Lisa Jean Taylor-Swanson, PhD, MAcOM
	Kai-Yin Hsu
	Joe Chang
	Lisa Ann Conboy

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September 12, 2017

Joerg Heber, PhD, Editor-in-Chief
PLOS ONE
1160 Battery Street
Koshland Building East, Suite 225
San Francisco, CA 94111
United States

Lisa Taylor-Swanson, PhD, MAcOM, EAMP
Assistant Professor
College of Nursing
University of Utah
10 South 2000 East
Salt Lake City, UT 84112

Dear Dr. Heber,

We would like to submit an article titled "Characteristics of Gulf War Illness participants in an acupuncture study" for peer review. This paper is relevant to *PLOS ONE* readers. Specifically, this paper confirmes an identified trend of veterans of the Gulf War having poorer health than those who served during the Gulf War era (but not in the Gulf War). This is a trend that has been followed for two decades, and our data confirm this knowledge. We believe *PLOS ONE* readers will find this article both relevant and useful.

As first author on the paper, I'll also serve as primary contact for the paper while in the review process. I look forward to working with you and your associates during this review process.

Respectfully yours,

Lisa Taylor-Swanson, PhD, MAcOM; EAMP

1   **Title:** Characteristics of Gulf War Illness participants in an acupuncture study

2   **Running Title:** Characteristics of GWI participants

10

11   **Competing Interests**: The authors have no competing interests to disclose.

12

13   **Key words:** Gulf War Illness, veteran characteristics, pain, sleep, mood

14

15   **Abstract**

16   BACKGROUND: Gulf War veterans reported significantly more often

17   nonspecific multiple complex medical symptoms, including fatigue, sleep and mood

18   disturbances, cognitive dysfunction, and musculoskeletal pain.

19   METHODS: We analyzed baseline characteristics reported by Gulf War Illness

20   (GWI) study participants. The data was from a pragmatic randomized clinical trial to

21   test the effects of individualized acupuncture treatments offered in extant

22   acupuncture practices in the community. Veterans with diagnosed symptoms of GWI

23   were included in the trial. This report focuses on sociodemographic characteristics,

24   the SF-36 physical component scale score (SF-36P), and the McGill Pain scale at

25   baseline.

26   RESULTS: Of the 192 participants assessed for eligibility, 104 subjects

27   underwent randomization. Mean age was 48 years, 13% were female and over 2/3

28   self-described white in both groups. Over 2/3 reported diseases of the

29   musculoskeletal system and connective tissue and over half reported symptoms,

30   signs and abnormal clinical or laboratory findings. Over 1/3 of participants indicated

31   that they were currently diagnosed with anxiety (36% of the sample), depression

32   (35%) and Post Traumatic Stress Disorder (33%). Other concurrent diagnoses

33   included sleep apnea (28%) and Chronic Fatigue Syndrome (28%).

34   DISCUSSION: Gulf War Veterans in the present study diagnosed with Gulf War

35   Illness report many similar symptoms and diagnoses to GW veterans studied

36   elsewhere. Specifically, increased mental health conditions such as depression,

37   anxiety, and PTSD were identified in this sample, as well as physical conditions of

38   Chronic Fatigue Syndrome and unidentified symptoms. Safe and effective

39   interventions for these symptoms and condition need to be identified and studied for

40   this population.

41   CONCLUSIONS: Further research is needed to identify and test safe and

42   effective interventions for symptoms and conditions experienced by Gulf War

43   veterans.

44

45   **Introduction:**

46   Gulf War veterans have experienced poorer health than Gulf Era veterans

47   (veterans who served during the same time-period as the Gulf War but not deployed

48   to the Gulf War itself). Gulf War veterans' health was examined in 1995 (1) to study

49   veterans' health 5 years after the war, in 2001 to examine veterans' health a decade

50   after the war (2, 3), and again in 2012-13 (4) to examine veterans' health 20 years

51   after the war. Five years after the war, Gulf War veterans reported a multitude of

52   health issues with higher prevalence than Gulf Era veterans, including unexplained

53   symptoms and medical conditions, increased healthcare utilization and functional

54   impairment. Gulf War veterans also reported poorer general health than non-

55   deployed veterans. (1) A decade after the war, GW veterans were more likely to

56   report persistently poor health, chronic fatigue, new onset of poor health and chronic

57   disease. GW veterans' health worsened in comparison with nondeployed veterans.

58   Twenty years after the war, GW veterans reported a significantly higher prevalence

59   of physical and mental health conditions compared to Gulf Era veterans. (4) These

60   conditions included Gulf War Illness, Chronic Fatigue Syndrome, neuralgia, gastritis,

61   chronic obstructive pulmonary disease, fibromyalgia, tachycardia, dermatitis,

62   rheumatoid arthritis, seizures, coronary heart disease, migraine headaches,

63   hypertension and asthma. Statistically significantly higher rates of mental health

64   conditions in Gulf War Veterans compared to Gulf War era veterans included Post

65   Traumatic Stress Disorder, major depressive disorder, anxiety disorder, and high

66   somatic symptom severity. Gulf War veterans that rated their health as excellent

67   (3.6%) or very good (10.3%) was significantly lower (p<0.001) than the proportion of

68   Gulf Era veterans who reported their health as excellent (6.1%) or very good

69   (16.2%).

70   The CDC defined GWI as having at least one chronic symptom from two of the

71   following three areas: mood/cognition, fatigue, and musculoskeletal. (5) GWI is also

72   a chronic multisystem condition that is significantly associated with deployment to the

73   Gulf War. (6)

74   Although no specific disorder has been identified in GWI veterans, and the

75   etiologic basis and clinical significance of their symptoms remain unclear(6), recent

76   GWI research studies have elucidated the mechanisms of GWI. Our primary

77   objective was to characterize self-reported survey findings among GWI veterans who

78   met our case definition and participated in this study to add additional information to

79   the literature on the topic of Gulf War veteran health and disease.

80

81   **Methods**:

82   We analyzed baseline characteristics reported by GWI study participants. The

83   data was from a pragmatic randomized clinical trial (NCT01305811) to test the

84   effects of individualized acupuncture treatments offered in extant acupuncture

85   practices in the community. (7) Veterans with diagnosed symptoms of Gulf War

86   Illness were included in the trial. Full trial design details have been previously

87   published (8) and Institutional Review Board (IRB) review and approval was given by

88   the XX Review Board on September 4, 2009. This report focuses on baseline

89   sociodemographic characteristics, secondary diagnoses, types of diseases, the SF-

90   36 physical component scale score, SF-36 Fatigue scale (9), McGill Pain scale (10),

91   Measure Yourself Medical Outcome Profile (MYMOP) (11), Primary Care Evaluation

92   of Mental Disorder (12), Multidimensional Assessment of Fatigue (13), The Profile of

93   Mood States (14), Pittsburg Sleep Quality Index (15), Beck Anxiety Inventory (16),

94   Carroll Depression Scale (17), Social support, Social Networks, and Stress (18, 19).

95   Missing data were coded as missing completely at random (MCAR), under the

96   assumption that omissions happened completely at random. All statistical analyses

97   were performed with SAS software (SAS Institute Inc, 2010), version 9.4 (SAS

98   Institute, Cary, NC).

99   **Results**:

100   Of the 192 participants assessed for eligibility, 104 subjects underwent

101   randomization. Fifty-two were randomized to biweekly treatment and 52 were

102   randomized to waitlist to weekly treatment. Mean age was 48 years in both groups,

103   13% were female in both groups, and 83% self-reported as white in the biweekly

104   treatment group and 79% self-described as white in the waitlist to weekly treatment

105   group. Baseline characteristics are summarized in Table 1.

106

107   [Please insert Table 1 here]

108

109   Participants were asked about their concurrent secondary diagnoses. Over 1/3

110   of participants indicated that they were currently diagnosed with anxiety (36% of the

111   sample), depression (35%) and Post Traumatic Stress Disorder (33%). Other

112   concurrent diagnoses included sleep apnea (28%), Chronic Fatigue Syndrome

113   (28%), Gastroesophageal reflux (19%), Irritable Bowel Syndrome (13%),

114   Fibromyalgia (9%), Other diagnoses (7%) and Interstitial Cystitis (1%).

115

116   [Insert Table 2 about here]

117

118   Major types of diseases experienced by participants were queried by listing ICD-

119   10 diagnoses. Diagnoses were then bundled and labeled by type of disease (listed

120   as either symptom 1 or symptom 2, combined here) and include Diseases of the

121   musculoskeletal system and connective tissue (75% of the sample reported

122   experiencing these diseases), Symptoms, signs and abnormal clinical and laboratory

123   findings, not elsewhere classified (51%) and Mental, Behavioral and

124   Neurodevelopmental disorders (31%).

125

126   [Please insert Table 3 about here]

127

128   Baseline scores for measures of physical function, fatigue, mood states,

129   interpersonal support, optimism, locus of control, social support, body

130   consciousness, catastrophizing, depression and anxiety are noted in Table 4.

131

132   [Please insert Table 4 here]

133

134   **Discussion**:

135   Participants in this study were predominately male, white, not Hispanic and the

136   average age was 48 years. Over a third of this sample reported current diagnoses of

137   anxiety, depression, PTSD. Over a quarter of the sample reported concurrent

138   diagnoses of sleep apnea and Chronic Fatigue Syndrome. Diseases of the

139   musculoskeletal system and connective tissue were reported by 75% of the sample,

140   symptoms, signs and abnormal clinical and laboratory findings, not elsewhere

141   classified were experienced by 51% of the sample, and Mental, Behavioral and

142   Neurodevelopmental disorders were reported by 31% of the sample. Ratings on

143   standardized instruments were higher than population norms for the SF-36 scale,

144   where 2005-2006 population mean= 50.68 (SD = 14.48) and our sample mean =

145   67.73 (SD = 23.48) (20). The present study participants also reported differently than

146   population norms on the Beck Anxiety Inventory. Population mean = 13.1 (SD =

147   10.97) (21) and the present study sample mean = 36.90 (SD – 10.97).

148   This study adds an additional perspective of Gulf War veterans who are

149   diagnosed with Gulf War Illness. Data were collected 2009-2012, roughly twenty

150   years after the Gulf War. Data reported here parallel prior studies of Gulf War

151   veterans, specifically that this sample all experienced Gulf War Illness, and both

152   studies report Gulf War veterans with higher levels of mental health conditions

153   including PTSD, anxiety and depression.   Neuralgia, fibromyalgia and migraine

154   headaches were reported in another study (ref 2) and in this sample, over 75% of

155   participants reported musculoskeletal pain. We cannot draw exact comparisons due

156   to the heterogeneity of questions being asked, but the present study parallels other

157   findings regarding pain experienced by Gulf War veterans. Chronic Fatigue

158   Syndrome is another parallel between the study by Dursa EK, Barth SK,

159   Schneiderman AI, Bossarte RM. et al (2016) and the present study.

160   There are limitations to the present study. All participants were drawn from the

161   New England area, and these data are not necessarily transferrable to other regions

162   of the USA. Second, we sampled only Gulf War veterans diagnosed with Gulf War

163   Illness. It is plausible that our sample was 'sicker' than the general Gul War veteran

164   population. Last, we sampled people with GWI who were interested in participating in

165   a trial of acupuncture for GWI. It is plausible we have a skewed sample, as well.

166   It is clear that veterans of the Gulf War are more likely to experience mental

167   illness such as depression, anxiety and PTSD, as well as physical illnesses and

168   symptoms, as compared to veterans of the same era who did not serve in the Gulf

169   War. Further research is needed to identify safe and effective interventions for

170   veterans of the Gulf War.

171

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229

Table 1

Table 1. Baseline Characteristics of the Study Population. Baseline Characteristics of the Study Population. Standard Deviations (SD) are offered for age, baseline pain, and baseline Sf-36(P).

Characteristic	Biweekly Treatment (N = 52)	Waitlist to Weekly Treatment (N = 52)
Age-year +/- SD	48.2 +/-9.9	48.2 +/- 3.5
Female sex-N(%)	7 (13%)	7 (13%)
Self reported Race-N(% of total)		
White	43 (83%)	41 (79%)
Black or African-American	5 (0.1%)	5 (0.1)
Asian	1 (0.02%)	0
American Indian/Alaskan Native	0	1 (0.02%)
More than one race	0	2 (0.04%)
Other	3 (0.06%)	3 (0.06%)
Self reported Hispanic N(% of total)		
Yes	2 (0.04%)	4 (0.08%)
No	48 (0.92%)	46 (0.88%)
No answer	2 (0.04%)	2 (0.04%)
Baseline Pain N(group mean) +/- SD	50(29.5) +/- 8.5	45 (29.8) +/-8.9
Baseline Sf-36(P) N(group mean) +/- SD	51 (67.7) +/-24.6	49 (66.4)+/-24.7

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Table 2

Table 2. Frequency of secondary diagnosis

Secondary Diagnosis	Total N	Yes – I have it currently N (%)	Yes – I had it in the past N (%)	No – I have never had it N (%)	I'm not sure N (%)	Missing N (%)
Fibromyalgia	103	9 (8.74%)	2 (1.94%)	63 (61.17%)	13 (12.62%)	16 (15.53%)
Interstitial Cystitis	103	1 (0.97%)	0 (0%)	73 (70.87%)	10 (9.71%)	19 (18.45%)
Chronic Fatigue	103	28 (27.18%)	8 (7.77%)	40 (38.83%)	14 (13.59%)	13 (12.62%)
Gastroesophageal reflux	103	20 (19.42%)	13 (12.62%)	43 (41.75%)	9 (8.74%)	18 (17.48%)
Sleep apnea	103	29 (28.16%)	3 (2.91%)	44 (42.72%)	10 (9.71%)	17 (16.50%)
Depression	103	36 (34.95%)	18 (17.48%)	36 (34.95%)	6 (5.83%)	7 (6.80%)
Anxiety	103	37 (35.92%)	15 (14.56%)	33 (32.04%)	10 (9.71%)	8 (7.77%)
IBS	103	13 (12.62%)	5 (4.85%)	49 (47.57%)	14 (13.59%)	22 (21.36%)

PTSD	103	34 (33.01%)	9 (8.74%)	31 (30.10%)	18 (17.48 %)	11 (10.68%)
Other	103	7 (6.80%)	0 (0%)	23 (22.33%)	7 (6.80%)	66 (64.08%)

Table 3

Table 3. Major Symptoms

2016 ICD-10-CM Codes	Sx 1 Percent (%)	Sx 2 Percent (%)	Total Percent (%)
Mental, Behavioral and Neurodevelopmental disorders	16.5	14.56	31.06
Diseases of the nervous system	2.91	4.85	7.76
Diseases of the eye and adnexa	0.97	0	0.97
Diseases of the ear and mastoid process	0.97	0	0.97
Diseases of the digestive system	1.94	8.74	10.68
Diseases of the musculoskeletal system and connective tissue	43.69	32.04	75.73
Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified	26.21	25.24	51.45
Injury, poisoning and certain other consequences of external causes	0.97	0	0.97
Unknown	5.83	14.56	20.39
Total	100	100	200

Table 4. Baseline Measures

Measure	Average Score both groups Mean (SD); N
Physical Functioning (SF-36 Physical Component)	67.73 (23.48) N=87
Summary Fatigue Score	29.01 (7.47) N=84
Mood states (POMS sum)	112.73 (22.88) N=63
Interpersonal social support (ISEL sum)	35.46 (7.72) N=91
MYMOP Score	4.84 (0.88) N=86
Optimism	27.13 (7.60) N=91
Locus of control-Internal	24.51 (4.98) N=96
Locus of control -Chance	16.76 (5.13) N=95
Locus of control-Powerful	15.54 (4.93) N=94
People social support (SOC)	2.26 (0.53) N=94
Body Consciousness	16.46 (4.08) N=94
Catastrophizing	17.79 (7.68) N=92
Whitely Depression	9.90 (1.91) N=88
Beck Anxiety	36.90 (10.97) N=83
Depression Score (CDEP sum)	7.15 (1.10) N=94
Factor of Depression -Guilt	6.78 (0.75) N=93
Factor of Depression –Work Interests	6.15 (1.02) N=92
Factor of Depression -Retardation	6.77 (0.92) N=91
Factor of Depression - Agitation	6.71 (1.08) N=91
Factor of Depression –Psychological Anxiety	6.29 (1.19) N=90

Effectiveness in Clinical Trial Recruitment: An Analysis of Methods used in a Novel Randomized Clinical Trial for the Treatment of Gulf War Illness

Matthew J. Hadfield[1], OMS-IV, Ann Barbett MAci[3] Lisa Conboy, ScD[2,3]

Author Affiliations:
1. Liberty University College of Osteopathic Medicine
2. Beth Israel Deaconess Medical Center, Harvard Medical School
3. The New England School of Acupuncture at MCPHS University

Corresponding Author:

Dr. Lisa Conboy
Beth Israel Deaconess Medical Center, Harvard Medical School

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Introduction:

Available data indicates that following the first Persian Gulf War (Operating Desert Shield/Storm, occurring 1990-1991) a quarter of veterans returning were found to experience a symptom cluster, that later became known as Gulf War Illness [1,2]. There are an estimated 100,000 veterans that have GWI. This symptom cluster included chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome and digestive complaints. Additionally, these veterans have reported mood-related psychiatric disorders including depression, anxiety, and post-traumatic stress disorder (PTSD) [3]. GWI illness has been poorly understood with no clearly delineated pathophysiologic process since it was first defined by the Centers for Disease Control (CDC) in 1995. Due to the nature of the disease, the presentation from one patient to the next is often highly variable making successful treatment with conventional pharmacologic strategies.

Methods:

A Department of Defense (DoD) funded randomized-controlled clinical trial was conducted evaluating the effectiveness of acupuncture in the treatment of Gulf War Illness. This was carried out through the utilization of private practice acupuncture offices dispersed throughout

southern New Hampshire and Massachusetts. Further details on the design of the trial [4], and positive results of the trial are published elsewhere.

Recruitment Strategies and Goals:

In the original design and planning of the study, recruitment would rely heavily on the Gulf War Illness Registry, a registry constructed by the Department of Veterans Affairs to track GWI cases. Unfortunately, access to this registry was revoked just prior to the onset of patient recruitment. This created a relatively large roadblock in recruitment as the study team would no longer have direct access to the patient population in question. Several challenges arose from losing access to the GWI registry. First and foremost, the study was geographically restricted to New England. With no database to refer to, it was difficult to determine how many Gulf War veterans were in this region of the United States. It was also impossible to assess what percentage of Gulf War veterans located in New England were suffering from Gulf War Illness.

Recruitment methods were quickly redirected towards mass media efforts to reach as many Gulf War veterans as possible. Initially, mass mailings were conducted and were aimed at organizations that frequently had contact with veterans. The purpose of these mailings was to introduce these organizations to the study and to ask for assistance in veteran outreach.

The goal of recruitment was 120 subjects. At the nine-month mark, the study team had managed to consent and enroll only 16 study participants. Re-evaluation of recruitment tactics at that point led to the application for veteran information from the Defense Manpower Data Center, an organization with a large veteran database. They provided very basic metrics that proved invaluable in improving veteran recruitment into the study. Because of this newly acquired information, we could apply for a grant extension to continue enrollment for six additional months.

Study recruitment lasted 22 months from August 2010 to June 2012. The total number of veterans enrolled was 104. Results of the clinical Trial were published in 2016 by authors Conboy, et al.

Methods:

Media – Due to the novel nature of the trial, several interviews were conducted by the principle investigator of the study, Lisa Conboy PhD, with publications such as the Boston Herald, Boston Globe, Newton Tab, Springfield Republican and Stars and Stripes. These interviews occurred between 2010 and 2012, and garnered attention about the work being done and assisted with patient recruitment. The study PI was interviewed on Rt. 9 Veterans Forum Cable Access television program in July of 2011. Advertisements were also placed in the Boston Metro, a free newspaper distributed at metro stations and various locations around the greater Boston area. Advertisements also appeared on Somerville, Massachusetts's local cable access station.

Radio advertisements were placed on WRKO/WEEI (local talk and sports radio programming) in 2011. Each advertisement was 30 seconds in duration and a total of 17 were run. The study PI interviewed on WRKO AM talk radio in Boston, MA and WADK in Newport, RI. Both interviews occurred in 2011.

Mass Transit – A full color advertisement measuring 11' x 27' was placed on Boston Metro trains running on the 'red' line train service. This line was strategically chosen based on the areas the line serviced: Cambridge and downtown Boston. The line ran from Mattapan, Ma to

Braintree Ma. The advertisement ran from 12/21/2010-01/14/2011. The dates were chosen in a fashion that would allow veterans who may have been traveling during the holidays to still be exposed to the advertisement and inquire about the study.

Internet – Brief, text only, advertisements were listed on several websites that were strategically chosen for a high likelihood of exposure to Gulf War veterans. These web pages included clinicaltrials.gov, craigslist, clinicalconnections.com, and gulfweb.org. The study was also promoted on the New England School of Acupuncture website.

Veteran specific websites including 91outcomes.com and veteransnewsnow.com also featured study related information on their web pages.

Events:
The study team attended a total of five Yellow Ribbon Post Mobilization seminars to reach veterans returning from deployments. The goal was to reach out to veterans that may have served in the first Gulf War and to spread awareness about the trial. Flyers were also handed out for display in National Guard barracks and the offices of several veteran centered agencies attending these events.

A Disabled American Veterans (DAV) conference was attended in 2011 to increase study awareness in the disabled veteran population.

Flyers, Letters and Post Cards:
The study team utilized 8.5 x 11' flyers printed in color for distribution. These posters featured pertinent information for potential study participants including symptoms of Gulf War Illness, requirements pertaining to deployments (dates, times, locations), and contacts for inquiring about more study information. The flyers were printed in two different formats. The first format was in the form of a letter containing all information for the study. The second format was printed with information and pull-away tabs at the bottom of the flyer that a person could take with them. These tabs had contact information for the study.

Multiple methods were employed to disseminate the flyers. A mass mailing sent to personnel at employment and career centers throughout the Commonwealth (Department of Employment and Training, Commonwealth of Massachusetts.) These centers were chosen to hopefully get information to veterans that may have been seeking employment. It is unknown if these flyers were posted or distributed in any way.

Flyers were also posted in areas felt by the study team to have a high likelihood of being seen by a veteran or the family member/friend of a veteran. These sites included Massachusetts Bay Transportation Authority (MBTA) stations, Veterans of Foreign Wars (VFW) posts, veteran centered agencies and private businesses. These flyers were placed by paid work study students. Flyers were also placed in the registration bags that were distributed at a Healthy Living Expo occuring in the greater Boston area.

Flyers were distributed to all acupuncturists affiliated with the study to display in their offices and to distribute to any individuals who inquired. As the study progressed, flyers were also given to study participants to distribute to their acquaintances and to post in their local communities.

Letters were sent to pain management clinics in the greater Boston area but no responses were ever received.

Letters and postcards were also sent directly to veterans using address information we had acquired. This was anticipated to be the most effective way to reach veterans and to recruit them for the study. Nine separate mass mailings were conducted with 2,481 total letters being distributed. Mailings were grouped based on towns/cities and their distance from Newton, Massachusetts (where the main study would be conducted) and their distance to participating acupuncture offices. Cities and towns closest to Newton were targeted first. Mailings were spaced out over 2-4 weeks to allow adequate time for potential participants to be appropriately screened and processed without overwhelming the study team.

After the initial letters were sent, the study team utilized the mailing of 4,000 postcards that were mailed in batches of 200-400 every 3-4 weeks. We continued to expand our network of affiliated acupuncturists and our mailing areas to reach more potential participants. Whenever a post-card or letter failed to reach an address and was returned to sender, this was noted in a Microsoft Excel spreadsheet. Participants that went through a screening process were also noted in the Excel spreadsheet. This database was utilized to ensure no one would receiving multiple mailings.

3,244 contacts were utilized from the data we had available and of these 984 (29%) were addresses that yielded undeliverable mail. 500 names from the manpower data were cold called to increase exposure to the study, of these only 3 study subjects were acquired.

Data Collection:

Follow up to inquiries was returned with an initial screening call, conducted by trained study staff members. These study members followed a script of open ended questions to learn how the individual learned about the study. A database was constructed in Filmmaker pro to track information provided by callers. This database tracked how the person learned about the study, results of their initial screening for the study, the results of their medical screening for the study and if they were ultimately enrolled or not. The data regarding how participants learned about the study is listed in table 1.0. Participants that stated they got a card in the mail were added to the Manpower data category as that is the pool of addresses we used for our mass mailings. The categories were the result of our standardized question of "how did you learn about this study." No category was created for participants who learned about the study from multiple, overlapping sources.

Advertising Costs:

Advertising costs for the Boston Metro totaled $2,224 and included seven ads that ran for six weeks. This form of advertising generated 4 total study participants (cost per participant being $561.00.) Ads run on clinicalconnection.com totaled $207 and lead to three study participants (total cost per participant $207.) Sports radio ads on WRKO Boston/WEEI Worcester cost a total of $833 dollars and generated three study participants (278$ per participant.) Many participants had difficulty remembering how they were initially exposed to the study, making tracking which method was most effective more difficult.

Results:

The recruitment effort with the highest yield for generating study participants was recruitment aimed directly at veterans from the Manpower Data Center information. We were privileged to contact information for 3,390 veterans residing in the Commonwealth of Massachusetts. Of that number, it determined that 3,244 veterans would fit our criteria in terms of location and being able to participate in the study. These 3,244 veterans received postcards and flyers. The second highest source of study participants came from flyers/posters distributed at veteran's associations and agencies.

One study tactic was attempting to have study participants that were having a positive experience with the study recruit other veterans. A total of six study participants were recruited from word of mouth referrals.

Through all recruitment efforts that were employed we generated sixty e-mail inquiries about the study and 225 phone calls. 163 of these inquires made it through to an initial study screening interview (the others were deemed to be disqualified upon initial contact with the study team.) The initial screening process was conducted over the phone and consisted of a checklist that was based on the original GWI diagnostic criteria set forth by the Centers for Disease Control. These criteria included: 1. Deployed to the "Gulf Theater of Operations" as defined by 38 CFR 3.317 which included Iraq, Kuwait, Saudi Arabia, Oman, the Persian Gulf, the Arabian Sea and the Red Sea as well as the airspace above these regions in the years 1990-1992. 2. They have at least two of the symptoms from the three CDC clusters of symptoms. 3) These symptoms have lasted longer than six months in duration. Each symptom cluster must be characterized as "mild-moderate" or "severe" with a least one symptom in each cluster having the designation as "severe."

Information from the phone screening was logged into the Filemaker Database. This database automatically determined the eligibility of each veteran to enter the study based on the inputted information. Eligible veterans were asked if they would like to participate in the study and those who wished to move forward were scheduled for an in person medical screening performed by the study physician. Medical screenings were conducted on one to two Saturdays each month depending on the physician's availability.

Of the 225 initial calls that were placed, 29 veterans declined to participate in the study. The reasons for these included being too far from the study location, lack of reimbursement for travel, and inability to commit from a time standpoint. Of the 163 veterans who went through the pre-screening, 22 were found to be ineligible for the study. Five eligible veterans chose not to move forward with the medical screening. The remaining 136 participants moved on to the medical screening stage of the recruitment process.

15 medical screening appointments that were arranged resulted in a "no-show" by the potential study participant. In these cases, the veteran was contacted by the study team but contact could not be established. 49 veterans went through the study to some degree but were lost throughout the study and could not be reached. The results of the study were a successfully recruited population of 104 subjects (90 men and 14 women.) The average study participant age was 48 years old. 17 people removed consent from the study either for personal reasons.

Discussion:

The most successful recruitment strategy for the study was directed posters/flyers/phone calls for the population we were attempting to recruit. These efforts proved to be far more effective than blindly advertising in public areas. It is also likely (although difficult to quantify) that the

synergistic effects of veterans getting exposed to the study through multiple channels increased their likelihood to inquire.

Several unexpected hurdles were encountered throughout the study. The internet proved to be a very effective medium for garnering inquiries about the study. However, due to the indiscriminate nature of web searches, the study team received inquiries from all over the country. In most of these instances, it was not possible for these veterans to participate in the study.

Other road blocks stemmed from staffing issues related to the study. Since there was only one study physician it was necessary to work around his schedule to conduct medical screens. In some instances, veterans, would need to wait 4-6 weeks to get a medical screen conducted. Increased time between study inquiry and medical screening results in a lesser likelihood of the veteran showing up to the appointment.

The study also had difficulties with veterans not showing up to appointments for medical screenings. The study team would call the week prior to the appointment but this did not always ensure the veteran would still make it. This resulted in more phone calls to reschedule and an increased need for manpower to logistically handle the rescheduling.

Through the duration of the entire study there were two instances where the price of gas rose above $4.00/per gallon. This was an issue raised by the participants and impacted study recruitment and compliance. In the future, more budgeting should be delegated to transportation costs as this proved to be a large hurdle for veteran participation in the study.

Tables/Figures:

Source of Inquiry	No. of Responses
Boston Herald	5
Boston Metro	4
Clinical Connection.com	1
Friend	10
Internet Search	1
Manpower Database	103
MBTA	2
National Gulf War Resource Center	1
NESA	1
Other	19

Other Veteran Agency	12
Radio (WRKO)	3
Springfield Republican	5
Stand Down Event	1
Stars & Stripes	1
Unknown	3

a. General Inquiries

Source of Inquiry	No. of Responses
Boston Herald	4
Boston Metro	1
Friend	6
Internet Search	1
Manpower Database	65
MBTA	2
NESA	1
Other Veteran Agency	12
Radio (WRKO)	3
Springfield Republican	4
Stand Down Event	1
Stars & Stripes	1
Unknown	3

b. Sources by Study Subject

Number of Subjects	Race	Percentage	
83	Caucasian	79.8	
10	African American	9.6	
4	Hispanic/Latino	3.8	
1	Asian	1.0	
1	Native American	1.0	
4	Other	3.8	

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Title: Matrix analysis of traditional Chinese medicine differential diagnoses in Gulf War Illness

Running title: Chinese Medicine differential diagnoses in GWI

Lisa Taylor-Swanson, Joe Chang, Rosa Schnyer, Kai-Yin Hsu, Beth Ann Schmitt, Lisa Conboy

Affiliations:

Lisa Taylor-Swanson, PhD, MAcOM, EAMP University of Washington, School of Medicine

Joe Chang, LicAc. Duke University Department of Community and Family Medicine

Rosa Schnyer, DAOM, University of Texas, School of Nursing

Kai-Yin Hsu, LicAc, MAOM, New England School of Acupuncture, at MCPHS.

BethAnn Schmitt, Lic Ac, MEd.,MAOM, New England School of Acupuncture, at MCPHS.

Lisa A. Conboy, ScD, MA, MS, New England School of Acupuncture

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Abstract

Objective: To qualitatively categorize Chinese Medicine (CM) differential diagnoses in a study of acupuncture for veterans with Gulf War Illness (GWI).

Subjects and Methods: We randomized 104 veterans diagnosed with GWI to a 6-month acupuncture intervention that consisted of either weekly or bi-weekly acupuncture treatments. CM differential diagnoses were recorded at baseline and at 6-months. These CM diagnoses were evaluated using Matrix Analysis to determine patterns of excess, deficiency and channel problems. These diagnoses were examined within- and between-person to determine patterns of change and stability in CM diagnoses over time.

Results: Diagnoses of excess combined with deficiency decreased from 43% at baseline to 39% of the sample at 6-months. Excess + deficiency + channel issues decreased from 26% to 17% while deficiency + channel decreased from 11% to 4% over the study duration. There is a trend of decreased numbers of veterans with all three types of differential diagnosis combinations. This would suggest that fewer people were diagnosed with concurrent excess, deficiency and channel issues and perhaps lessening in complexity of their presentation. These findings are preliminary given the sample size and the amount of missing data at 6-months.

Conclusion: This first step to organize and define CM differential diagnoses is novel both in the context of GWI and in the context of other conditions as CM differential diagnoses have not been examined by using Matrix Analysis previously. Given the complexity and bothersomeness of GWI, and that veterans' health is worsening over time, attention must be given to how to best provide effective care for veterans with GWI. This work may inform future directions, such as the development of a CM manual.

Introduction

According to clinical and registry programs, 25% of the 700,000 veterans of the first Gulf War are affected by multiple symptoms and co-morbid medical diagnoses that include: chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, arthralgia, digestive complaints, and mood-related psychiatric disorders, including depression, post-traumatic stress disorder (PTSD), and other anxiety disorders (Conboy, St John, & Schnyer, 2012). Some first Gulf War veterans have been diagnosed with Gulf War Illness (GWI). GWI presents with difficult symptom presentations, often with complex co-morbid symptoms that consist of fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain. These symptoms are grouped into three symptom clusters by the Centers for Disease Control and Prevention (CDC): fatigability (fatigue 24 hours or more after exertion), mood and cognition (feeling depressed, irritable, anxious, difficulty in concentrating, problems getting to sleep), and musculoskeletal (joint or muscle pain). Overall, veterans diagnosed with GWI are stable (not improving) at 5- and 10-year follow ups (Steele, Sastre, Gerkovich, & Cook, 2012) and no standard of care presently exists (Conboy et al., 2016). There is a need for innovative and effective care of GWI.

Acupuncture has been shown to be effective in treating some of the symptoms of GWI as evident in published research studies, specifically for pain, anxiety, depression, and musculoskeletal disorders. The parent study to this project demonstrated clinically and statistically significant improvement in SF-36 physical and McGill Pain Index scores in the group receiving acupuncture twice per week for six months (Conboy et al., 2016). Preliminary studies indicate that acupuncture may be effective in the management of other complex diseases which share a similar cluster of symptoms (Conboy et al., 2016). The complex diagnostic and treatment process of Chinese medicine (CM), which is tailored to each individual's clinical presentation, can provide an effective framework for evaluating and addressing the complex symptoms presented in GWI. Currently, there are no theoretical CM frameworks for GWI. Our

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team set out to evaluate the theoretical CM framework through a matrix analysis of CM differential diagnoses in our GWI acupuncture study.

The present study is a secondary data analysis of differential diagnoses routinely recorded at baseline and 6-months in the parent study. We sought to better understand if there were subgroups within this sample in terms of CM differential diagnoses.

Methods

Quantitative Parent Study

Detailed methods are described elsewhere, but a brief summary of the parent study is provided here for context (Conboy et al., 2016). We randomized 104 participants from VA centers around Boston and provided individualized acupuncture care in private practices by experienced Licensed Acupuncturists. Participants provided informed consent and were randomized either to bi-weekly acupuncture for six months or a 2-month wait-list, after which they received weekly acupuncture for four months. This research was approved by New England Institutional Review Board and United States Army Human Research Protection Office. All participants continued to receive standard care as needed.

The acupuncture intervention lasted approximately one hour and consisted of consultation with the Licensed Acupuncturist, assessment of the participant, and the development of an individualized treatment plan. Acupuncture points were chosen according to CM differential diagnosis; acupuncture needles were inserted and retained for 30-45 minutes.

Qualitative Sub-Study

The data were drawn from the parent study and participant data was included if available and there were no exclusion criteria. Descriptive statistics were therefore drawn from the parent study, as all participants were included in this study.

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Frequency counts were tabulated for each differential diagnosis. Matrix analysis was then applied to categorize differential diagnoses according to categorizations of excess, deficient and channel disorders for baseline and 6-months visits. This allowed the detection patterns of consistency and patterns of change in CM differential diagnoses over time.

Matrix Analysis

Matrix analysis is a qualitative data analysis that involves "the crossing of two or more main dimensions . . . to see how they interact" (Miles & Huberman, 1994). Here, we have two dimensions: individual differential diagnoses and categories of excess, deficiency and/or channel diagnoses. These categories are not mutually exclusive and commonly co-occur in clinical practice. Matrices can be descriptive (depicting conditions), outcome-oriented (depicting results or consequences), or process-oriented (depicting dynamics of change) (Averill, 2002). Here we develop both descriptive and process-oriented matrices.

Two coders (LTS and JC) were trained and all participants' data were coded first according to each type of differential diagnosis, and then according to excess, deficiency and/or channel concerns. Cohen's kappa was calculated and the two coders compared any differences, and discussed their interpretations of the coding scheme until agreement was obtained.

Results

The study participants were not statistically significantly different from one another with respect to the following demographics: the mean age was 48.2 in both groups, 13% were

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female in both groups, 83% were self-reported as white in the biweekly treatment and 79% in

the wait-list to weekly treatment group.

Table 1. Baseline Characteristics of the Study Population. Baseline Characteristics of the Study Population. Standard Deviations (SD) are offered for age, baseline pain, and baseline Sf-36(P).

Characteristic	Biweekly Treatment (N = 52)	Waitlist to Weekly Treatment (N = 52)
Age-year +/- SD	48.2 +/-9.9	48.2 +/- 3.5
Female sex-N(%)	7 (13%)	7 (13%)
Self reported Race-N(% of total)		
White	43 (83%)	41 (79%)
Black or African-American	5 (0.1%)	5 (0.1)
Asian	1 (0.02%)	0
American Indian/Alaskan Native	0	1 (0.02%)
More than one race	0	2 (0.04%)
Other	3 (0.06%)	3 (0.06%)
Self reported Hispanic N(% of total)		
Yes	2 (0.04%)	4 (0.08%)
No	48 (0.92%)	46 (0.88%)
No answer	2 (0.04%)	2 (0.04%)
Baseline Pain N(group mean) +/- SD	50(29.5) +/- 8.5	45 (29.8) +/-8.9
Baseline Sf-36(P) N(group mean) +/- SD	51 (67.7) +/-24.6	49 (66.4)+/- 24.7

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Reliability

Data were coded by two people (JC & LTS) with master's degrees in CM and a

combined twenty-three years' clinical experience. Reliability was calculated with Cohen's kappa.

The reliability coefficient obtained after coders were trained was .93.

Matrix Analysis

Participants' differential diagnoses were noted at the baseline and 6-month visit.

Participants' differential diagnosis consisted of one to six diagnoses (e.g., *concurrent* Spleen Qi

deficiency and Liver Qi constraint and stagnation in the channels). Frequencies of each

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differential diagnosis component at baseline are displayed below in Table 1. Next, each diagnosis was organized according to CM theory into one of three categories. These categories are general or overarching theoretical constructs, and encompass deficiency (e.g., insufficient Qi), excess (e.g., overabundance of Qi, which then may become stagnant) and pathology of the acupuncture meridians and channels (e.g., Qi is stagnant in a channel, causing local pain). The categories and the associated differential diagnoses are:

Deficiency (of Qi, Blood, Yin or Yang)

Excess (of Damp, Heat, Cold, Phlegm, Fire, Yang rising, Wind, Qi stagnation or Blood stagnation)

Channel pathology (Qi & Blood stagnation in the channels, Deficiency of Blood, Yin in the channels, 8 Extraordinary Meridians or Damp Bi Syndrome).

We further analyzed the top 10 of 17 categories with Matrix Analysis, according to frequency. The 10 highest frequency categories are: Qi deficiency, Blood deficiency, Yin deficiency, Qi stagnation, Dampness, Heat, Yang rising, Phlegm, Qi & Blood stagnation and Damp Bi syndrome, as noted in Table 1.

Table 1 Differential diagnosis categories at baseline

Category	N=
DEFICIENCY	
Qi	67
Blood	24
Yin	33
Yang	7
EXCESS	
Damp	25

Heat	23
Cold	2
Phlegm	11
Fire	1
Yang Rising	13
Wind	8
Qi Stagnation	51
Blood Stagnation	10
CHANNEL	
Qi & Blood stagnation	30
Deficiency	2
8 Extraordinary Vessels	4
Damp Bi Syndrome	13

Each participant's differential diagnosis was then categorized as to whether there was a single type of pathology (e.g., only deficiency, or only excess, or only channel pathology - no combination of the categories) or co-occurring differential diagnoses categories (e.g. combination of excess and deficiency, or excess and channel issues, or deficiency and channel issues; or all three, with excess and deficiency and channel issues all co-occurring). These co-occurrences of diagnosis categories are noted in Table 2.

Table 2 Co-occurrence of differential diagnoses at baseline

Category	N=
Only 1 category (e.g., excess or deficiency or channel)	12
Excess + Deficiency	40

Excess + Deficiency + Channel	24
Deficiency + Channel OR excess + channel (NOT excess and deficiency)	16
No data available	2

One category of differential diagnosis

Twelve individuals had a single category of differential diagnosis. Of the participants with a single category of diagnosis, Qi deficiency was the most common (6 instances), followed by Yin deficiency, Yang rising, Damp Bi syndrome (2 instances), Yang deficiency, Blood deficiency, Qi stagnation and channel Qi stagnation (1 instance each).

Two categories of differential diagnoses

Forty participants' differential diagnoses were a mixture of excess and deficiency. The most frequent differential diagnosis combination was Qi deficiency & Qi stagnation (n=12 cases), followed by Qi & Yin deficiency with Qi stagnation (n=3 cases), Qi deficiency with Damp & Heat (2 cases), Qi deficiency with Qi stagnation, Damp & Heat (2 cases), Blood deficiency with Qi stagnation, Damp & Heat (2 cases), Qi & Blood deficiency with Qi stagnation, Damp & Heat (2 cases), Qi, Blood & Yin deficiency with Qi stagnation & Yang rising (2 cases). Fourteen (14) other combinations were each seen in one case only. One case could not be summarized in the table (see note).

Three categories of differential diagnoses

Twenty-four participants presented with differential diagnoses at baseline categorized by co-occurring excess and deficiency and channel pathology. These participants were the most complex in their differential diagnoses, with multiple co-occurring categories of disharmony. Please refer to Table 3.

Table 3 Participants with three categories of differential diagnoses: co-occurring deficiency and excess and channel pathology

Category	N=
1 deficiency, 1 excess, 1 channel (5 cases)	
Qi xu; Qi stagnation; **Qi/blood stagnation**	2
Qi xu; Damp; **Damp Bi**	1
Yin xu; Qi stagnation; **Damp Bi**	1
Qi xu; Qi stagnation; **Damp Bi**	2
1 deficiency, 2 excess, 1 channel (2 cases)	
Qi xu; Damp, Phlegm; **Qi/Blood channel**	1
Qi xu; Qi stagnation, Damp; **Qi/Blood channel**	1
1 deficiency, 4 excess, 1 channel (1 case)	
Qi xu; Qi stag, Damp, Heat, Phlegm; **Qi/Blood channel**	1
2 deficiency, 1 excess, 1 channel (3 cases)	
Qi, Blood xu; Yang rising; **Qi/Blood channel**	1
Blood, Yin xu; Yang rising; **Qi/Blood channel**	1
Qi, Yin xu; Qi stagnation; **Damp Bi**	1
2 deficiency, 2 excess, 1 channel (3 cases)	
Blood, Yin xu; Qi stag, heat; **Qi/Blood channel**	1
Qi, Yin xu; Qi stag, Heat; **Qi/Blood channel**	1
Qi, Yin xu; Heat, Phlegm; **Qi/Blood channel**	1
2 deficiency, 3 excess, 1 channel (4 cases)	
Qi, Yin xu; Damp, Heat, Phlegm; **Qi/Blood channel**	1

Qi, Yin xu; Qi stag, Damp, Heat; **Qi/Blood channel**	1
Qi, Yin xu; Qi stag, Heat, Yang rising; **Damp Bi**	1
Qi, Blood xu; Qi stagnation, Damp, Heat; **Qi/Blood channel**	1
3 deficiency, 1 excess, 2 channel (1 case)	
Qi, Blood, Yin xu; Damp; **Qi/Blood channel, Bi syndrome**	1
3 deficiency, 3 excess, 1 channel (1 case)	
Qi, Blood, Yin xu; Heat, Yang Rising, Phlegm; **Qi/Blood channel**	1
NOTE: n=4 cases' data not summarized in Table 6 as one of their differential diagnosis categories was not a top 10 in frequency.	

Matrix analysis at 6-months

Differential diagnoses were evaluated at 6-months and classified according to excess, deficiency and channel problems. Please refer to Table 4. A within-person analysis was also performed and 25 participants' diagnoses changed from one category to another (e.g., co-occurring excess and deficiency at baseline and one category only at 6-months), 51 participants' category was the same at baseline and at 6-months, and 18 participants could not be categorized due to missing data.

Table 4. Baseline and 6-month differential diagnoses

Category	Baseline Frequency N (%)	6-month Frequency N (%)	Descriptive changes
Only 1 category (e.g., excess or deficiency or channel)	12 (13%)	15 (16%)	Slight increase
Excess + Deficiency	40 (43%)	36 (38%)	Slight decrease

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Excess + Deficiency + Channel	24 (26%)	16 (17%)	Decrease
Deficiency + Channel	10 (11%)	4 (4%)	Decrease
Excess + Channel	6 (6%)	5 (5%)	Slight decrease
No data available	2 (1%)	18 (20%)	Increase

Discussion

The results of the matrix analyses indicate that Gulf War Illness (GWI) is rather complex in terms of CM diagnoses and presentation. The symptom presentations of GWI demonstrated co-occurrences of both deficient and excess as the most frequent both at baseline (43% of the sample) and at 6-months (38% of the sample). Three categories co-occurring, namely excess and deficiency and channel pathology was the next most frequent category both at baseline (26%) and 6-months (17%).

Regarding the single category of diagnosis: Qi deficiency was the most frequent type of deficiency, and this can progress with further depletion or stress to include concurrent Yin or Blood deficiency. Chronic stress and/or environmental exposures (such as the Gulf War veterans were exposed to) may lead to Yang rising, Dampness or Qi stagnation which are common differential diagnoses of symptoms like pain, headache, and emotional issues.

Regarding two categories of diagnosis: Qi deficiency and Qi stagnation co-occur frequently and this is commonly seen in clinic. Qi deficiency can lead to Qi stagnation, and the formation of Dampness, generation of internal Heat and Phlegm. Yang can become unrooted without the Yin and Blood to anchor it, and this leads to Yang rising (we see it co-occurs with Qi and Blood deficiency, and Yin deficiency).

Regarding three categories of diagnosis with co-occurring excess, deficiency and the addition of channel issues. Clinically, we would interpret long-standing Qi deficiency and Qi stagnation to engender Dampness, Phlegm, internal Heat, Dampness, and, eventually Qi/Blood

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stagnation in the channels and Damp Bi syndrome. Here, participants exhibited a superficial excess condition, with underlying deficient symptoms, which leads to symptoms within the channels of pain (qi & blood stagnation and damp-bi syndrome).

It is interesting to note that participants in the bi-weekly acupuncture treatment group demonstrated clinically and statistically significant improvement in SF-36 Physical and McGill Pain scores (Conboy et al., 2016) and yet the overall trend is stability in differential diagnoses. In CM theory, a concept about root and branch presentations may help explain why symptoms improved, but differential diagnoses did not always change. It is plausible that the differential diagnoses were reflecting root presentations, meaning very stable individual constitutions. This root is compared to branch diagnoses, which are reflective of emergent or acute conditions. For this reason, it is plausible that while participants' symptoms improved, their differential diagnoses were rather stable, perhaps reflective of root constitutions and not branch symptoms.

Limitations of this study include an increase in missing data at 6-months. This made it impossible to categorize 18 participants' differential diagnoses. Additionally, while the parent study was adequately powered to detect clinically and statistically significant change in primary and secondary outcomes, the analyses here demonstrated very small N in several classification categories. Due to the small sample size, the matrix analyses needs to be replicated with a larger GWI acupuncture study in order to further validate the differential diagnosis co-occurrences for GWI.

Since GWI is a unique and novel disease state and treatment with acupuncture has only initially been evaluated (our parent study), the development of a CM treatment manual is warranted. The treatment manual could utilize the differential diagnose framework developed here (deficiency, excess, channel concerns) as a basis for symptom presentations that are most likely to occur in patients with GWI. A theoretical CM pathological foundation could also be explored as to the causes of qi, yin, yang, and blood deficiency or excess in GWI patients.

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Future research that could also explore the initial GWI matrix analyses includes a comparative analyses of symptom clusters of menopausal patients with GWI and fibromyalgia patients. It would be of interest to explore these disease states as GWI, menopausal, and fibromyalgia patients often present similar symptom clusters (chronic fatigue, anxiety, and depression).

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The Importance and Determination of Dosage in Acupuncture Treatment

Introduction:
Gulf War Illness (GWI), or chronic multisymptom illness (CMI), is a complex illness characterized by multiple symptoms, including fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain, which are unexplained by physical and laboratory examinations. There is no standard of care treatment for this syndrome at this time. First defined by the Centers for Disease Control and Prevention (CDC) after the first Gulf War[i], it is commonly seen with a highly individualistic presentation, associated with clusters of symptoms and co-morbid medical diagnoses, including chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, arthralgia, digestive complaints, and mood-related psychiatric disorders, including depression, posttraumatic stress disorder (PTSD), and other anxiety disorders[ii,iii]. It has been shown to be remarkably stable at 5- and 10- year follow-ups[iv,v]. Of the 700,000 service personnel deployed to the Persian Gulf, at least one-fourth of the veterans of the first Gulf War (Operation Desert Shield/Storm, years 1990-1991) are affected by GWI[vi,vii]. GWI is twice as prevalent in deployed veterans, and seen in 15% of non-deployed veterans[viii]. CMI symptoms have been studied in cohorts of veterans in the United Kingdom, Canada, and Australia. The etiology of CMI is still unknown, and hypotheses involving exposures to vaccines, medications, pesticides, chemical munitions, and inhalation of depleted uranium dust and smoke from burning oil fields, have all been investigated.

In 2016, our study team published results of The Effectiveness of Acupuncture in the Treatment of Gulf War Illness. This pragmatic Randomized Clinical Trial tested the effects of individualized acupuncture treatments offered in extant acupuncture practices in the community; practitioners had at least 5 years of experience plus additional training provided by the study. Veterans with diagnosed symptoms of Gulf War Illness were randomized to either six months of biweekly acupuncture treatments (group 1, n = 52) or 2 months of waitlist followed by weekly acupuncture treatments (group 2, n = 52). Measurements were taken at baseline, 2, 4 and 6 months. The primary outcome is the SF-36 physical component scale score (SF-36P) and the secondary outcome is the McGill Pain scale. Of the 104 subjects who underwent randomization, 85 completed the protocol (82%). A clinically and statistically significant average improvement of 9.4 points (p = 0.03) in the SF-36P was observed for group 1 at month 6 compared to group 2, adjusting for baseline pain. The secondary outcome of McGill pain index produced similar results; at 6 months, group 1 was estimated to experience a reduction of approximately 3.6 points (p = 0.04) compared to group 2. (Conboy, 2016).

This parent trial used individualized acupuncture protocols. To better understand effective dosage of acupuncture for GWI symptoms, this paper considers the relationship between actual dose and symptom relief, as well as other factors related to actual dose which may have clinical importance.

Background:
Acupuncture is a widely used and increasingly studied treatment modality, yet substantial disagreement remains about its effectiveness and how it should best be utilized. Great progress has been made in elucidating the physiological effects of Acupuncture treatment, but there is still much that is only partially or poorly understood. (Zhuang, Xing, Li, Zeng, & Liang, 2013) There

are also a very wide range of treatment modalities and protocols that are used in Acupuncture treatments. For example, there are different styles of treatment (Chinese, Japanese, Korean etc.) as well as different treatment methodologies (5 elements, 8 principals, etc.) within each style. (O'Connor & Bensky, 1981)(Maciocia, 2008) These approaches vary greatly in terms of specific Acupuncture points used, and amount and type of stimulation. These differences need to be taken into account when comparing treatment effectiveness.

One of the important aspects of any medical procedure is dosage. Dosage is typically defined as quantity of treatment over time; i.e. how much, how often, and for how long is a treatment to be administered. Defining dosage is straightforward in the case of standardized medications based on patient's bodyweight or very clearly defined procedures, however the definition becomes more complex in the case of therapies such as Acupuncture which by nature may be non-standardized.

Many attempts have been made to define Acupuncture dosage, but to date there has been no universally adopted definition. In their discussion of this issue White et.al. defined a dose of Acupuncture treatment as "The physical procedures applied in each session, using one or more needles, taking account of the patients resulting perception (sensory, affective, and cognitive) and other responses (including motor). The dose may be affected by the state of the patient (e.g. nervous, immune and endocrine systems); different doses may be required for different conditions." (White et al., 2008)

In their paper on Acupuncture dosage and dysmenorrhea Armor and Smith (Armour & Smith, 2016) make a distinction between "neurophysiological dose" and "cumulative dose". *Neurophysiological dose* variables include number of needles, Acupuncture points used, retention time, and mode of stimulation (including needle manipulation and Electro-acupuncture, as well as adjunctive techniques such as cupping, and press tacks.) *Cumulative dose* refers to the total number and frequency of treatment sessions. The authors also mention the potential importance of treatment timing, i.e. when in the time course of an illness should Acupuncture best be used.

The first definition (White et al., 2008) is comprehensive, but too general and descriptive to result in anything more than defining a dose of Acupuncture as a single Acupuncture treatment. The second set of definitions for *cumulative dose* (Armour & Smith, 2016) are more specific and therefore more clinically useful, and it is this definition that we will use to frame the definition for Acupuncture dosage in this paper.

In an effort to simplify meaningful data collection, many Acupuncture studies have deliberately chosen to treat conditions and/or use treatment paradigms which administer the same neurophysiological dose for all patients. Unfortunately, this type of study design may often interfere with the clinical effectiveness of Acupuncture treatment, and may be of limited value since it does not reflect how Acupuncture is usually practiced. (Liu et. al 2015, MacPherson, et. al. 2007)

Acupuncture as it is actually practiced clinically is an individualized treatment process, with neurophysiological dosage determined by the patient's condition on the day of treatment. (O'Connor & Bensky, 1981)(Maciocia, 2008)(Langevin & Schnyer, 2017) Since the neurophysiological dose will vary between patients and from treatment to treatment, even patients who have been treated for the same condition using the same cumulative dosage of treatments, are unlikely to have received the same neurophysiological dosage of Acupuncture. This situation can lead to confusion as to how total Acupuncture dosage over time should be compared across patients.

However, if a consistent diagnostic and treatment process is followed in determining optimal neurophysiological dosages for each patient, meaningful comparisons about cumulative dosage and treatment effectiveness can be made. This approach has been followed in a number of recent studies. (Schnyer, Iuliano, Kay, Shields, & Wayne, 2008) (Conboy, St John, & Schnyer, 2012) (Conboy et al., 2016) In all of these studies the participating Acupuncture practitioners were trained and experienced in using the same treatment protocols, and they agreed to use the same specifically outlined diagnostic procedures in deciding how to treat their patients. Within this constraint they were free to choose whatever specific neurophysiological dose that they felt was appropriate for a patient on a given day. This process reflects the way that Acupuncture is actually practiced in most clinics. Studies designed in this way are both naturalistic and rigorous, conforming to the STRICTA recommendations for Acupuncture controlled trials. (MacPherson et al., 2002)

Once the issue of neurophysiological dosage is addressed, it becomes meaningful to compare cumulative dosages. Currently there is surprisingly little data about the importance of cumulative dosage in determining Acupuncture treatment effectiveness. A literature review was done by the author (BAS) in September 2017 searching Medline, Google scholar, and Cochrane databases using the keywords 'Acupuncture therapy' (MeSH term) AND 'frequency,' 'dosage' and 'schedule'. No comprehensive studies or reviews of the significance of cumulative dosage across all types of Acupuncture treatment were found.

However, there are numerous studies containing some cumulative dosage data, and some of these studies include data comparing the effectiveness of different cumulative dosages for specific conditions. A 2005 study investigating Acupuncture treatment for fibromyalgia indicated that cumulative dosage was more important than correct Acupuncture point needle placement in determining treatment success, and that 3 treatments weekly provided greater pain relief than 1 treatment weekly. (Harris et al., 2005) A large study investigating Acupuncture treatment of chronic pain indicated that both number of needles used, and total cumulative dosage were significant; with more needles and more treatments associated with greater pain relief. (MacPherson et al., 2013) Indeed our 2016 study investigating the treatment of Gulf War Illness with Acupuncture found that treatment twice weekly was more effective than treatment once weekly. (Conboy et al., 2016)

Armor and Smith did a literature review to specifically investigate the relationship between Acupuncture treatment dosages and pain relief outcomes in treating dysmenorrhea. Summarizing the results of 11 trials, they concluded that treatment timing, needle stimulation, and number of needles all appeared to be significant factors in influencing pain reduction. Treatment started

before the onset of menses appeared to produce the greatest pain reduction during menses, one needle appeared to be more effective than many for pain reduction during menses, but conversely more needles used before menses was most effective. Regarding cumulative dosage, comparisons between studies with varying cumulative dosage amounts and treatment frequencies did not indicate any clear dosage responses, however unfortunately none of the studies undertook direct comparisons of treatment frequency or total number of treatments. The authors concluded that future studies should include these comparisons in the study design. (Armour & Smith, 2016)

A 2012 Chinese review article on Acupuncture cumulative dosage summarized a number of small un-blinded trials examining varying cumulative dosages for different conditions. These preliminary studies indicated that the optimal cumulative dosages are different for different conditions; varying from 2 treatments per day for stroke patients, to 1 treatment every 3 days for neurologic pain, to 1 treatment weekly for some chronic conditions.("Research on the Effect that Frequency Do to the Curative Effect of Acupuncture--《Journal of Liaoning University of Traditional Chinese Medicine》2012 年 10 期," n.d.)

Traditional Acupuncture conventions regarding ideal cumulative dosage amounts differ considerably. In China, patients are typically treated 3 or more times weekly, for at least a month, often more. (O'Connor & Bensky, 1981) In the West the norm is more likely to be 1 or perhaps 2 treatments weekly, typically for 6 to 10 weeks, although this will vary considerably depending upon the practitioner and the economic circumstances of the patient. In China, patients often get both a larger and a more concentrated cumulative dose than in the West. Frequently, in both Western and Chinese Acupuncture texts, no justifications other than historical convention or specific clinical case studies are given for choosing specific cumulative dosage amounts.(O'Connor & Bensky, 1981)("Research on the Effect that Frequency Do to the Curative Effect of Acupuncture--《Journal of Liaoning University of Traditional Chinese Medicine》2012 年 10 期," n.d.)

Methods: In 2009, our study team was awarded a three-year Congressionally Directed Medical Research Program grant to implement the RCT "The Effectiveness of Acupuncture in the Treatment of Gulf War Illness" (W81 XWH). This pragmatic Randomized Clinical Trial tested the effects of individualized acupuncture treatments offered in extant acupuncture practices in the community; practitioners had at least 5 years of experience plus additional training provided by the study. Veterans with diagnosed symptoms of Gulf War Illness were randomized to either six months of acupuncture treatments twice per week (group 1, n=52) or 2 months of waitlist followed by weekly acupuncture treatments (group 2, n=52). Measurements were taken at baseline, 2, 4 and 6 months. The primary outcome is the SF-36 physical component scale score (SF-36P) and the secondary outcome is the McGill Pain scale. In addition we collected data on other symptoms, psychosocial health, alliance with practitioner, and medical history[ix]. Of the 104 subjects who underwent randomization, 85 completed the protocol (82%). A clinically and statistically significant average improvement of 9.4 points (p=0.03) in the SF-36P was observed for group 1 at month 6 compared to group 2, adjusting for baseline pain. The secondary outcome

of McGill pain index produced similar results; at 6 months, group 1 was estimated to experience a reduction of approximately 3.6 points (p=0.04) compared to group 2.[x]

This current analysis considers the relationship between actual dose, as measured by practitioner treatment records, and our main outcomes using Pearson correlation. Secondly, considering dose in a manner similar to adherence, we consider if there is a relationship between actual dose and other physical and psychosocial health markers at baseline, as well as quality of relationship with practitioner.

When considering which variables to consider in the prediction of adherence, we first thought of confidence, and/or satisfaction with treatment. But in this sample there is very little variation in these variables and all subjects reported high confidence and satisfaction. Thus instead we considered the baseline variables of health behaviors (smoking, & drinking behavior), anxiety (Beck), depression (Whitely), social support (ISEL), mood (POMS) and reported social networks. We also considered the patients' viewpoint on the quality of the patient-practitioner relationship at the first measurement timepoint of 2 months; we did not measure relationship at baseline because the patient-practitioner relationship had not started yet.

We considered the relationships between actual dose and the variables above with descriptive statistics (mean, variance) and simple Pearson correlations for continuous variables and Spearman correlation for categorical variables.

Results:

Actual dose/number of treatments across the whole sample ranged from a minimum of one treatment to a maximum of 49, with a mean of 23.99 (SD 12.98). As expected the treatment arm assignment is related to actual dose (r=0.73, p<0.001).

Figure 1:Frequency distribution of actual dose by treatment group assignment.
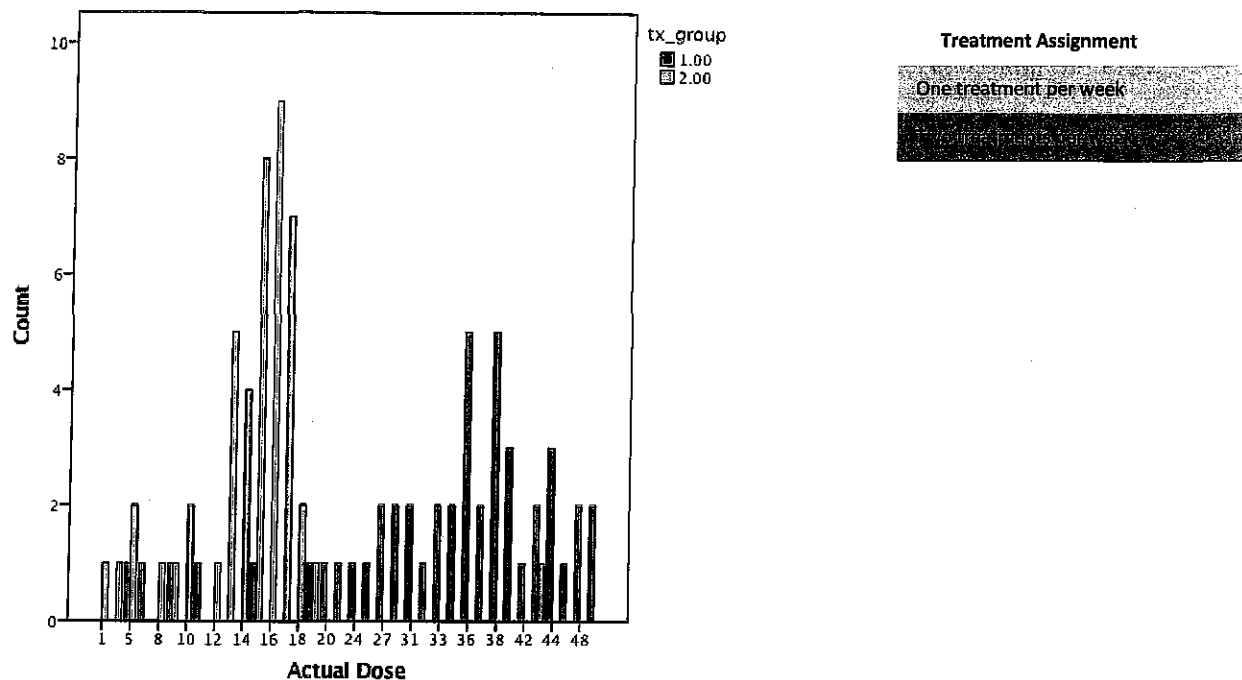
Actual Dose

We found no statistically significant relationships between any of the psychosocial or behavioral variables at baseline and actual dose; that is there was no indication that a certain type of subject is more likely to adhere.

We did find that actual dose was positively and significantly (to $p < 0.05$) related to Working Alliance Inventory (WAI) factor of *bond* ($r=0.26$, $p<0.02$) with practitioner. The other two WAI factors, shared *tasks* ($r=0.18$, $p<0.1$), and *goals* ($r=0.21$, $p<0.06$) of patient-practitioner relationship quality were marginally significant (to $p<0.1$). That is, at 2 months a better patient-practitioner relationship (at least from the patients' point of view), predicts higher therapy usage. By 4 months these relationships became more significant. Working Alliance Inventory (WAI) factor of *bond* ($r=0.29$, $p<0.01$), *tasks* ($r=0.23$, $p<0.01$), and *goals* ($r=0.25$, $p<0.03$).

Regarding symptom reporting, actual dose was not related to baseline levels of pain or SF-36-physical function, but was related to symptom improvement by study completion.

Table 1: Pearson correlation of pain and function with actual dose at baseline, and change scores from baseline to 6 months.

	Baseline		Change Baseline to 6 months	
	Pain	Physical Function	Pain	Physical Function
Actual Dose Pearson Correlation	.085	-.121	-.252*	.301**
Sig (2-tailed) N	(0.441) 85	(0.257) 90	(0.030) 74	(0.007) 80

Figure 2: Relationship of Actual Dose and pain resolution by 6 months. Change scores above zero indicate worsening reported pain; scores below the line indicate improvement in pain.
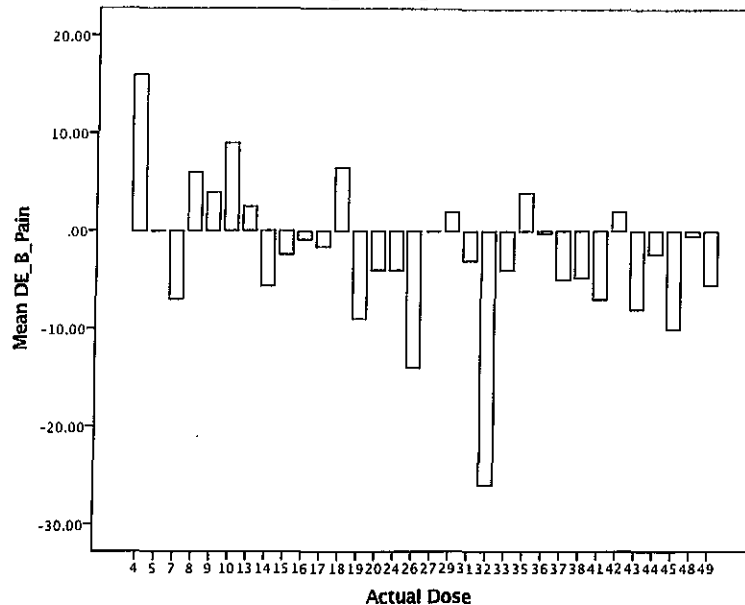
Figure 3: Relationship of Actual Dose and improvements in physical function by 6 months. Change scores below zero indicate worsening reported function; scores above the line indicate improvement in function.
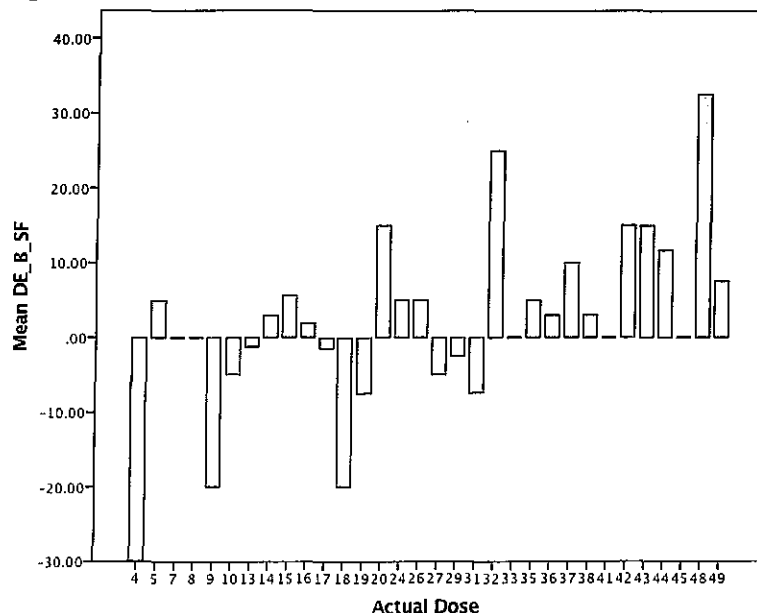
We also considered the relationship of Actual Dose and pain resolution by 6 months. (Figure 2). It appears that in this sample symptom improvement most clearly begins at 18 treatments. In consideration of the relationship of Actual Dose and physical function by 6 months. (Figure 3), it appears that in this sample symptom improvement most likely occurs after 33 treatments.

Conclusion:
In order for Acupuncture to be both as effective and as economical as possible, it is important to determine the ideal cumulative dosage and dosage schedules that should be used for a given condition or range of conditions. Future Acupuncture studies should include more comprehensive cumulative dosage comparisons in their study designs.

The literature reviewed indicate that cumulative dosage appears to be a significant factor in determining treatment success, and that this relationship is not necessarily a straightforward or linear one. However, the primary goal of many Acupuncture studies is to compare Acupuncture treatment effectiveness to sham treatment effectiveness. Therefore, cumulative dosage data is often not as comprehensive as it might be if the studies were designed the goal of learning the most appropriate dosage for a condition. In addition, many studies of Acupuncture are small and preliminary in nature, and many of them utilize low cumulative dosages across all subjects. (Liu et al., 2015)

In the case of GWI and individualized acupuncture, we found that actual dose was positively related to the symptom reduction, but not related to baseline severity of symptoms. We also considered factors that could influence adherence and found that within the confines of our RCT, actual dose was not related to baseline psychosocial characteristics of social networks, social support, anxiety, or depression; nor was actual dose related to previous use of acupuncture. Actual dose was related to the bond the patient felt with the practitioner at 2 months, and this relationship became more significant by the 4-month treatment time point. This finding suggests that patient's commitment to treatment early on in the study predicts adherence.

Limitations and future work
One weakness of this exploratory descriptive analysis is our use of multiple comparisons making the statistical significance of testing only suggestive. Our future work includes a factor analysis of baseline variables to determine a baseline factor structure to use for symptom resolution prediction. We found 5 factors and can look at relationship in subgroups

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Introduction:

The Therapeutic Alliance (TA) is the relationship created between patient and practitioner, which is also recognized as therapeutic relationship, therapeutic bond, treatment alliance, helping alliance, or working alliance. It is considered vital to treatment effectiveness in general psychotherapy (1-3) and is one of the most often studied subjects within concurrent clinical psychology (4-6). TA is rooted in brain coupling of patient and therapist (1) and purported to create the necessary climate and conditions in which other intervention contents can be successfully delivered by the therapist and absorbed by the patient (3,7-10). However, traditional TA is abraded by rapid pace of medical advances in addition to specialization and super-specialization (11)

TA encompasses three major elements: (i) tasks, the collaborative endorsement of the intra therapy activities (includes an understanding of what is required of each of the parties in the performance of these tasks, and an appreciation of the relevancy of the tasks to the therapy process); (ii) goals, the mutual agreement and valuing of the outcomes of the therapy; and (iii) bonds, that encompass the complex elements of attachment between the patient and clinician such as trust, empathy, personal liking and valuing (12).

Clinical studies have been reported by a meta- analysis that, the practitioner patient relationship has a significant effect on multiple healthcare outcomes including quality of life, psychological problems (e.g. anxiety, depression), re-consultation rate, smoking quit rate, pain relief, blood pressure, weight loss, etc. (13-26). TA consistently has shown to predict outcomes in psychotherapy (27-31) but studied to a lesser extent in other health fields such as Traditional Chinese Medicine (TCM) (32). TCM is a form of Eastern Medicine that is over 2000 years old. TCM practitioners utilize various techniques like acupuncture, tui na, nutrition, moxabustion, and tai qi to treat the mind and body of patients. Acupuncture being the most commonly used in America. In TCM, TA is a form of artistry, called the "Penetrating Divine Illumination" and is "the refinement of the physician's art." It is a transformative healing state between the Qi of physician and patient, which resonates at a spiritual level. Empathetic dialogue and assessment create the Penetrating Divine Illumination, which becomes part of the treatment or intervention. (33) Acupuncture, similar to other forms of health care areas, occurs in a specific setting that can affect clinical outcomes, the patient-acupuncturist relationship is one variable of this setting (34). Studies conducted in this area indicated TA as a predictive factor for therapeutic outcomes. Beneficial outcomes as a result of relationships between practitioners and clients in the field of

TCM have been reported (32). TA in acupuncture develops through the interaction between practitioner and patient in the initial health history intake and individualized treatment. (35) Indeed, acupuncture results are related to patient–practitioner relationship factors, not to placebo effects and patient expectations (36).

TA could be evaluated by Working Alliance Inventory (WAI). WAI has been used for measuring therapeutic relationship between the client and therapist and also predicting outcomes (37,38).

For the purpose of this study, we followed Miller's study, which modified the titles of therapists. We modified the title of therapists to Acupuncture Practitioner or Acupuncturist (32). Some studies evaluated TA from either the practitioner's or the patient's standpoint. However, we measured acupuncture-patient relationship from the standpoints of acupuncturists and patients concurrently (39). Since, TA is clearly a two-sided interactive process (40).

More patient-practitioner agreement on the quality of the patient-provider relationship, termed concordance, may be related to better clinical outcome (41, 42). In 2013, our study team completed a Phase II Randomized Controlled Trial (n=104) testing the effects of individualized acupuncture treatments offered to veterans in extant acupuncture practices (43, 44). The current study investigated the impact of concordance on therapeutic outcome of patients who received TCM treatment for Gulf War Illness. Gulf War Illness is a complex illness found among veterans of the first Gulf War, and characterized by multiple symptoms, including fatigue, sleep and mood disturbances, cognitive dysfunction and musculoskeletal pain.

Methods:

Horvath's Working Alliance Inventory (WAI) was used to measure TA (45-48). The WAI contains 36 items, with three subscales (Task, Goal, and Bond). Each item is scored on a 7-point scale ranging from 1 (never) to 7 (always) (49). The WAI has strong published support for reliability and validity (50). The WAI was administered to both subjects and clinicians at baseline, 2, 4, and 6 months of study exposure

Sample size:

The sample size per treatment group varied as only individual IDs with both baseline and endpoint measurements were utilized for change score analysis. For group 1, where acupuncture treatment was administered without delay and continued twice per week for a

6 month period, N=36 for ISEL_Sum, N=37 for CDEP_Depression_Sum and CDEP_Psychological_Anxiety_Sum. For treatment group 2, where treatment was delayed by 2 months and then continued weekly for the remaining 4 months, N=32 for ISEL_Sum, CDEP_Depression_Sum and CDEP_Psychological_Anxiety_Sum.
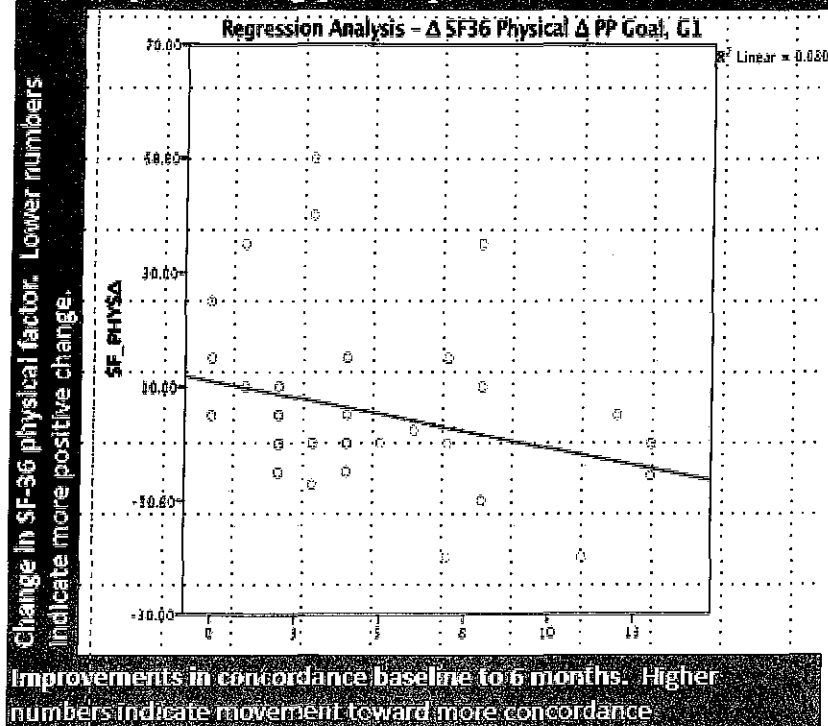
Statistical Analysis

Average WAI scores were calculated for each participant and practitioner and results for each factor were graphed by patient-practitioner dyad over time. We next calculated change in concordance for each dyad from baseline to 6 month endpoint. Linear regression models are used to measure the influence of degree of change in concordance on the change in outcomes of pain and physical function.

To assess whether a more congruent patient and practitioner relationship was indicative of superior treatment outcomes, Working Alliance Inventory-Short Revised (WAI-SR) difference scores between patients and practitioners were utilized and compared to clinical outcomes for ISEL_Sum, CDEP_Depression_Sum and CDEP_Psychological_Anxiety_Sum. WAI-SR difference scores were reviewed for similarity of agreement in tasks, goal and the formation of an affective bond during treatment.

Outcomes:

Over time, dyads reported increasingly positive scores on the WAI as the study progressed, and dyads moved toward higher levels of concordance. Regression analysis suggest that concordance across subscales results in improved outcomes. In particular, the factor of GOAL (shared treatment goals) was significantly related to improvements in physical functioning as measured by the SF-36 (to $p <= 0.05$) in both groups (Fig 1).

Figure 1. Clinical Improvement on the SF-36 physical factor by increasing patient-practitioner concordance

The correlation coefficients that were statistically significant (p<0.05) included:

Treatment group 2: CDEP_Psychological_Anxiety_1Sum Δ with PP_ Δ_Bond (p=0.009). Correlation coefficient analysis for ISEL_Sum and CDEP_Psychological_Anxiety_Sum varied, however, no stastistical significance was detected (Table 1).

Table 1. Pearson correlation coefficient per treatment group

Treatment Group 1						
Clinical Outcome	PP_Δ_Task	Sig. (1-tailed)	PP_Δ_Bond	Sig. (1-tailed)	PP_Δ_Goal	Sig. (1-tailed)
ISEL_1Sum (N=36)	-0.099	0.282	0.105	0.272	0.06	0.364
ΔISEL_Sum (N=36)	-0.051	0.385	-0.012	0.472	0.149	0.193
CDEP_Depression_1Sum (N=37)	-0.16	0.173	-0.21	0.106	-0.071	0.338
ΔCDEP_Depression_Sum (N=37)	0.029	0.432	0.035	0.418	0.085	0.309
CDEP_Psychological_Anxiety_1Sum (N=37)	-0.08	0.318	-0.205	0.112	-0.107	0.265
ΔCDEP_Psychological_Anxiety_Sum (N=37)	0.147	0.193	-0.02	0.453	0.117	0.246

Treatment Group 2						
Clinical Outcome	PP_Δ_Task	Sig. (1-tailed)	PP_Δ_Bond	Sig. (1-tailed)	PP_Δ_Goal	Sig. (1-tailed)
ISEL_1Sum (N=32)	-0.21	0.125	-0.081	0.33	-0.196	0.141
ΔISEL_Sum (N=32)	0.092	0.308	0.114	0.267	-0.037	0.421
CDEP_Depression_1Sum (N=32)	-0.044	0.406	-0.062	0.367	0.107	0.281
ΔCDEP_Depression_Sum (N=32)	0.114	0.267	-0.142	0.22	0.009	0.481
CDEP_Psychological_Anxiety_1Sum (N=32)	-0.214	0.12	-0.418	0.009	-0.103	0.287
ΔCDEP_Psychological_Anxiety_Sum (N=32)	0.117	0.262	-0.042	0.41	0.128	0.243

The correlation coefficients that were statistically significant (p<0.05) where:

Treatment group 1: Δ SF-36 Goal (p=0.046)

Treatment group 2: Δ SF-6 Task (p=0.042) and Δ SF-36 Goal (p=0.027) (Table 2).

Table 2. Pearson correlation coefficient per treatment group

Treatment Group 1						
Clinical Outcome	Task	Sig. (1-tailed)	Bond	Sig. (1-tailed)	Goal	Sig. (1-tailed)
Δ SF-36 (N=37)	-0.166	0.162	-0.269	0.054	-0.281	0.046
Δ McGill (N=37)	0.000	0.499	-0.221	0.095	0.044	0.399

Treatment Group 2						
Clinical Outcome	Task	Sig. (1-tailed)	Bond	Sig. (1-tailed)	Goal	Sig. (1-tailed)
Δ SF-36 (N=32)	-0.309	0.042	-0.158	0.195	-0.345	0.027
Δ McGill (N=31)	0.126	0.250	0.144	0.220	0.062	0.371

Conclusions

Analyses by dose of acupuncture and baseline factors are currently being performed.

These findings suggest that acupuncturists are skilled at gaining and improving therapeutic alliance. As with psychotherapy, we need to pay attention to nonspecific aspects of therapy because they can be active (51). The findings also show more concordance is related to better clinical outcome.

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RESEARCH ARTICLE

The Effectiveness of Individualized Acupuncture Protocols in the Treatment of Gulf War Illness: A Pragmatic Randomized Clinical Trial

Lisa Conboy[1]*, Travis Gerke[2], Kai-Yin Hsu[1], Meredith St John[1], Marc Goldstein[3], Rosa Schnyer[4]

1 New England School of Acupuncture, Newton, MA, United States of America, 2 Department of Epidemiology, College of Medicine and College of Public Health and Health Professions, University of Florida, Gainesville, FL, United States of America, 3 Boston Veterans Healthcare System/Jamaica Plain Campus, Boston, MA, United States of America, 4 University of Texas at Austin, Austin, TX, United States of America

* Lisa_conboy@hms.harvard.edu

Abstract

Background

Gulf War Illness is a Complex Medical Illness characterized by multiple symptoms, including fatigue, sleep and mood disturbances, cognitive dysfunction, and musculoskeletal pain affecting veterans of the first Gulf War. No standard of care treatment exists.

Methods

This pragmatic Randomized Clinical Trial tested the effects of individualized acupuncture treatments offered in extant acupuncture practices in the community; practitioners had at least 5 years of experience plus additional training provided by the study. Veterans with diagnosed symptoms of Gulf War Illness were randomized to either six months of biweekly acupuncture treatments (group 1, n = 52) or 2 months of waitlist followed by weekly acupuncture treatments (group 2, n = 52). Measurements were taken at baseline, 2, 4 and 6 months. The primary outcome is the SF-36 physical component scale score (SF-36P) and the secondary outcome is the McGill Pain scale.

Results

Of the 104 subjects who underwent randomization, 85 completed the protocol (82%). A clinically and statistically significant average improvement of 9.4 points (p = 0.03) in the SF-36P was observed for group 1 at month 6 compared to group 2, adjusting for baseline pain. The secondary outcome of McGill pain index produced similar results; at 6 months, group 1 was estimated to experience a reduction of approximately 3.6 points (p = 0.04) compared to group 2.

Conclusions

Individualized acupuncture treatment of sufficient dose appears to offer significant relief of physical disability and pain for veterans with Gulf War Illness. This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Gulf War Illness Research Program under Award No. W81XWH-09-2-0064. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

Trial Registration

ClinicalTrials.gov NCT01305811

Introduction

Clinical and registry programs indicate that 25% of the 700,000 veterans of the first Gulf War (Operation Desert Shield/Storm, years 1990–1991), are affected by clusters of symptoms and co-morbid medical diagnoses including chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, arthralgia, digestive complaints, and mood-related psychiatric disorders, including depression, posttraumatic stress disorder (PTSD), and other anxiety disorders [1,2]. Defined by the Centers for Disease Control and Prevention (CDC) [3], Gulf War Illness (GWI) is a complex, poorly understood illness, often with a highly individualistic presentation, and symptoms difficult for conventional medicine to treat effectively; GWI has been shown to be remarkably stable at 5- and 10- year follow-ups [1,4]. GWI is twice as prevalent in deployed veterans, and seen in 15% of non-deployed veterans [5]. There is no standard of care treatment for this syndrome at this time.

Although there are no published studies evaluating acupuncture's effectiveness in the treatment of GWI, acupuncture has been shown to successfully reduce many key symptoms of GWI including pain [5,6], musculoskeletal disorders [7,8,9], both acute and chronic pain after amputation in military contexts [10,11], fatigue [12], state, trait and situational anxiety [13], and depression [14,15,16]. Further, there is evidence that acupuncture may be effective in the treatment of other complex diseases such as irritable bowel syndrome [17], fibromyalgia [18], and post-traumatic stress disorder [19] and that acupuncture is well tolerated by patients, safe, and may be cost-effective compared to routine care [20].

Chinese Medicine, on which acupuncture is based, uses diagnostic and treatment procedures that are complex [21] and tailored to each individual's specific symptoms. Although this individualized treatment ideal is often replaced in clinical research with standardized protocols for the purposes of reliability and simplicity, the ability of Traditional Chinese Medicine (TCM) to be tailored to each patient is a core concept and strength that can be maintained successfully in a Randomized Controlled Trial (RCT) format [22]. This unblinded Phase II clinical trial utilized individualized treatment protocols, testing the effects of individualized acupuncture treatments offered in extant acupuncture practices.

Materials and Methods

Study design

Full trial design details have been published elsewhere [23]. Please see S1 File: Protocol. To maximize study compliance we employed acupuncturists to provide treatments in their own

offices in communities where veterans work and live. We began recruitment of subjects and practitioners with a catchment area of 30 miles from our research study offices, and widened our catchment area with increasing study duration.

Veterans with Gulf War Illness were randomized to either (1) acupuncture treatment twice per week for 6 months or (2) the wait-list comparison group consisting of usual care from baseline for 2 months, followed by weekly treatments for 4 months. We chose an active control group to maximize internal validity while allowing us to gather preliminary data on minimal effective treatment dose. The two month wait time allowed us to judge if GWI symptom presentations are stable in our sample, as has been shown in other GWI samples [1,5]. Our treatment schedule duration, dose, and specific Chinese Medicine techniques employed are based on the clinical experience of our expert practitioners, and informed by literature review. Details of the protocol and implementation were determined before the trial began via focus groups with senior acupuncture faculty at the New England School of Acupuncture.

The New England Institutional Review Board approved this research protocol on September 4, 2009. All human participants gave written informed consent. All of our study processes were approved and oversight is provided by: 1) The New England Institutional Review Board (http://www.neirb.com/), 2) United States Army Human Research Protection Office (https://mrmc-www.army.mil/rodorphrpo.asp). The study operated as planned between September 2009 and January 2013. Recruitment began immediately, and ran until July 2012. We initiated the clinical trial registration process before recruiting subjects, and registration was completed before we began to analyze the data. We confirm that all ongoing and related trials are registered. Please see S2 File. CONSORT Checklist.

Recruitment. We recruited via local advertisements and direct mailing to veterans of the first Gulf War drawn from the Defense Manpower Data Center (http://www.virec.research.va.gov/Non-VADataSources/DMDC.htm). Because the demographics of GWI veterans are unpublished, we did not know if there was a sufficient population near our study offices from which to draw our sample. Thus we designed the study to include treatment sites within a 100-mile radius of our study offices, and incorporated a mechanism to add treatment sites within that radius in areas where GWI veterans were found clustered. Thirty treatment sites were utilized. This design has the added benefit of allowing veterans to attain treatments near where they live and work, a technique that may have improved adherence.

Eligibility. All subjects met the illness definition of Gulf War Illness as determined by responses on the Gulf War Illness Symptom Checklist [5] and the inclusion/exclusion criteria set forth in the federal definition of Gulf War Illness as used for the Gulf War Registry (Please see Box 1). Subjects needed to pass through two eligibility screenings. First, a research assistant conducted a prescreening by phone, querying potential subjects about their illness and symptom experience. Second our study physician (MG) used the same criteria to complete an in-person medical screening. Our two-stage informed consent process included 1) verbal informed consent requested prior to the initial telephone screening, and 2) written informed consent administered in person, at the start of the screening visit. Please see Fig 1. An unblinded member of the study staff (LC) with no additional patient contact enrolled subjects. Study outcomes data were collected by electronic interface at our outpatient clinic. In fewer than 10% of cases, due to participants' time constraints, participants were allowed to take their surveys home to fill out, and then mail back. During this screening visit, participants chose their future treatment practitioner from a list of practitioners convenient for them.

Group assignment and interventions. Collaborator RD randomly assigned participants to the two study arms using permuted block randomization with variable block sizes and assignments provided in sequentially numbered opaque sealed envelopes. After baseline evaluation and consent, a member of the study staff without involvement in data collection with

Box 1: CDC Symptom Clusters for Gulf War Illness.

Inclusion:

1. deployed to the *"Gulf Theater of operations, as defined by 38 CFR 3.317, includes Iraq, Kuwait, Saudi Arabia, Bahrain, Qatar, the United Arab Emirates, Oman, the Gulf of Aden, the Gulf of Oman, the Persian Gulf, the Arabian Sea, the Red Sea, and the airspace above all of these locations"* in the years 1990–1,

2. have at least 2 of the following symptoms from the 3 CDC clusters of symptoms that have lasted for more than 6 months. Each symptom cluster must be characterized as "mild-moderate" or "severe" with at least one symptom in each cluster required to be severe.

A-Fatigability

- fatigue 24 hours or more after exertion

B-Mood and Cognition

- feeling depressed or
- feeling irritable or
- difficulty thinking or concentrating or
- feeling worried, tense, anxious or
- problems finding words or
- problems getting to sleep

C-Musculoskeletal

- joint pain or muscle pain

Exclusion:

Potential subjects will be excluded if they are:

- Currently enrolled in another clinical trial

- Have another disease that likely could account for the symptoms, as determined by our Medical Monitor

- Severe psychiatric illness (in the last 2 years psychiatric hospitalization, suicidal attempt, alcohol or substance abuse, use of antipsychotic medication) as measured by our primary screening instrument the Primary Care Evaluation of Mental Disorder (Prime MD).

- Unable to complete the protocol on based on the evaluation of the Medical Monitor

- Participants will not be excluded due to age, race, ethnicity, or gender limitations.

- Study is limited to United States veterans with Gulf War Syndrome, thus subjects could not be minors, illiterate, or unable to speak or understand English.
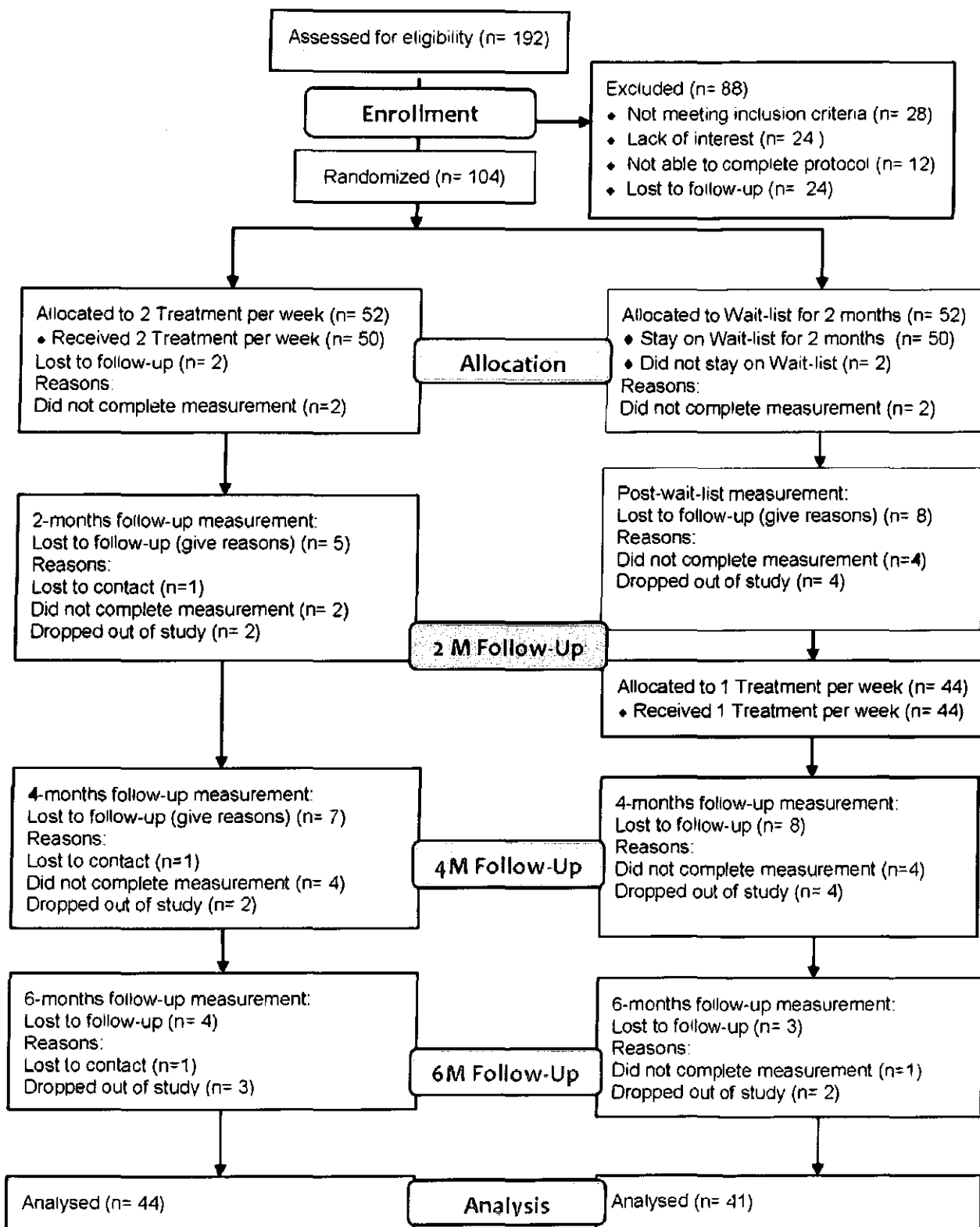
Fig 1. PLOS CONSORT Flow Diagram: Diagram of Screening, Randomization, and Follow-up.

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subjects (LC) opened the assignment envelopes and recorded the assignment of each participant in a confidential log. LC then reported to the subject's chosen practitioner the subject's contact information and study assignment. The chosen practitioner then took responsibility of contacting the subject to schedule and begin treatments. LC also called and/or emailed the subject to report if acupuncture treatments were to begin immediately (group 1), or in 2 months (group 2).

Licensed acupuncturists with at least 5 years of clinical experience, who received additional in-house training concerning GWI, provided the acupuncture treatments. Although there are many styles of acupuncture within Chinese Medicine, acupuncturists were chosen who self-reported use of the TCM model of diagnosis. During the first session, the acupuncturist conducted an interview reviewing the subject's medical history, symptoms and aspects of diagnosis from the perspective of TCM, including condition of the tongue, pulse, meridians, and acupoints. Each subject received an individualized diagnosis and treatment protocol addressing his or her unique pattern of symptoms. Brief interviews began each subsequent session, allowing patient and practitioner to prioritize symptoms, and identify any questions or concerns. Individualized treatment protocols allowed the practitioners to alter the treatment plan based on how the patient presented at the moment; including varying the selection of acupoints across treatments and adding particular co-interventions commonly used as part of TCM therapy to supplement manual needling (such as electroacupuncture for its efficacy in reducing pain and inflammation [24], heat therapies (e.g. heat lamp), Chinese massage, and press balls, tacks or magnets applied to points after needling). A sample treatment protocol is offered as S3 File.

Each session lasted approximately one hour. Acupoints were stimulated manually until "obtaining *de qi*," a technique characteristic of TCM to elicit a response felt by both the patient and the acupuncturist. This needling sensation, adjusted for the comfort and safety of each patient, may be experienced as a pinch that rapidly subsides, or a sense of spreading pressure, dull ache, or warmth. Needles were retained for 30–45 min (10–35 stainless steel, disposable needles per session). After needle insertion, subjects were left to rest or nap. The type of needle, including gauge [32–38] and length (15–50 mm) as well as the depth of insertion (subcutaneous to about 25 mm) varied according to the area of the body being treated (i.e. extremities vs. trunk). Choice of acupoints could vary during subsequent treatments to improve results. Herbs and supplements were not allowed. Subjects were encouraged (but not required) to remain with the same acupuncturist for the whole study period to allow for development of patient-practitioner rapport.

Outcomes. A single measure of severity that addresses all possible presentations of GWI does not exist. Thus we chose to use the SF-36, a 36-item, well-validated and reliable general measure of health [25]. Given the importance of function on quality of life, we focused the main outcome to the SF-36 physical component scale score (SF-36P). Similarly, as pain is a common to most GWI presentations and very relevant in veteran health, our secondary outcome is the McGill Pain scale, a 15- item measure recording participants' pain level and quality [26]. Outcomes were assessed with the assistance of a blinded study staff member at baseline, 2, 4, and 6 months. Raw data is offered as S4 File.

Subjects' confidence in and usability of acupuncture were measured in a few ways. Participants were asked (1) their confidence in recommending acupuncture to a friend or family member using a five point scale from "Very Confident" to" Not Confident", (2) the experience of the acupuncture using a five point scale from "Extremely Pleasant" to "Extremely Unpleasant", (3) the experience of their relationship with their practitioner on a five point scale from "Extremely Pleasant" to "Extremely Unpleasant", (4) how logical the acupuncture treatments were for them on a 6 point scale of "Very Logical" to "Not Logical at All".

Sample size

Our sample size was calculated to allow detection of clinically meaningful differences between treatment groups. Previous acupuncture research using our main outcome, the SF-36, in pain conditions [27,28] show a consistent standard deviation of 20 points in the SF-36 P for both baseline values and change scores. Sixty individuals per group (total n = 120) would offer us a power of 80% to detect the difference between groups of 7 points. Using Cohen's d estimation of effect size [29] a sample size of 60 would allow us to see a moderate effect. In further support of our main outcome, a 7.8-unit improvement has been estimated clinically relevant for patients with similarly serious conditions [30,31]. We estimated a dropout rate of 10%.

Statistical analyses

In our original proposal to the funder, to protect our main outcome from possibly large attrition, we proposed to initially test mean differences between groups following 2 months of treatment using Student's t-tests at an alpha = 0.05. Using this strategy, we observed a mean reduction in SF-36 for Group 1 of 0.32 versus a reduction 4.53 for Group 2 (p = 0.22).

Of more interest to the study team is what changes might be seen after the clinically informed 6 month treatment window. This 6 month analysis is done by author TG and investigates whether those subjects assigned to biweekly acupuncture experienced differences in the SF-36P score over follow-up compared to those subjects who received weekly acupuncture following a 2-month delay. To assess potential differences, generalized estimating equation (GEE) models were fit in order to account for the correlation induced by repeated measurements on each subject. Under the assumption that baseline McGill pain is prognostic for SF-36P over time, model adjustment was made for baseline pain to increase precision in the estimated parameters.32 Eight subjects did not report this baseline measurement and, under the assumption that missingness was completely at random, these subjects were not included in the analysis set. In summary, we estimated the GEE model

$$SF36 = \beta0 + \beta1 Itx + \beta2 It^2 + \beta3 It^4 + \beta4 It^6 + \beta5 \rho + \beta6 Itx It^2 + \beta7 It^4 + \beta8 Itx It^6$$

where Itx denotes an indicator for biweekly acupuncture; $It^2, It^4, and It^6$, are indicators for months 2, 4, and 6; ρ denotes baseline pain; and time was coded categorically to reflect suspected nonlinearities.

As a secondary analysis, the McGill pain score was similarly assessed for differences over time by treatment status. The time trend for the GEE fit was modeled categorically to account for nonlinear trends. The pain model was not adjusted for additional covariates, since no strongly prognostic variables were assumed to have been measured. All GEE models were fit using the software package geepack [33] in R version 3.1.1 under an exchangeable working correlation structure.

Results and Discussion

Study Population

Recruitment began in July of 2010 and the final follow-up visit was completed in January 2013. Please see Table 1 for baseline demographics and Fig 1. for study flow.

Outcomes

Of the 104 subjects who underwent randomization 103 completed at least one measurement timepoint, yielding 99.0% of data for analysis. However, 8 subjects are missing baseline pain data, yielding 95/104 = 91.3% in the analysis set. General Estimating Equations were used to

Table 1. Baseline Characteristics of the Study Population. Baseline Characteristics of the Study Population. Standard Deviations (SD) are offered for age, baseline pain, and baseline Sf-36(P).

Characteristic	Biweekly Treatment (N = 52)	Waitlist to Weekly Treatment (N = 52)
Age-year +/- SD	48.2 +/-9.9	48.2 +/- 3.5
Female sex-N(%)	7 (13%)	7 (13%)
Self reported Race-N(% of total)		
White	43 (83%)	41 (79%)
Black or African-American	5 (0.1%)	5 (0.1)
Asian	1 (0.02%)	0
American Indian/Alaskan Native	0	1 (0.02%)
More than one race	0	2 (0.04%)
Other	3 (0.06%)	3 (0.06%)
Self reported Hispanic N(% of total)		
Yes	2 (0.04%)	4 (0.08%)
No	48 (0.92%)	46 (0.88%)
No answer	2 (0.04%)	2 (0.04%)
Baseline Pain N(group mean) +/- SD	50(29.5) +/- 8.5	45 (29.8) +/-8.9
Baseline Sf-36(P) N(group mean) +/- SD	51 (67.7) +/-24.6	49 (66.4)+/- 24.7

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compare the group 1 vs. group 2 acupuncture subjects' experienced differences in the SF-36P, and McGill Pain scale.

Our analysis comparing baseline symptom levels to those at 6 months showed a significant average increase of 9.4 points in the SF-36 physical component scale score (SF-36P) for the biweekly acupuncture group at month 6 in comparison to the weekly waitlisted group, adjusting for baseline pain. Within the biweekly group, scores were generally stable at months 0, 2, and 4, with a mean increase of 6.6 points in SF-36P observed at month 6. A nonlinear pattern was observed for the waitlisted group, though a lower SF-36P relative to baseline was estimated for all subsequent months. Table 2 offers mean and empirical 95% confidence limits estimated for both SF-36P and McGill from 5000 simulations of the fitted GEE models, and Fig 2 provides a graphical summary of this information for SF-36P [34]. Though overlapping confidence bands are observed at time points determined significantly different in the GEE fit, we note that the simulation-based approach, while useful for visual interpretation, relies on slightly different calculations than the GEE model itself.

The secondary outcome of McGill pain index produced similar results. A decreasing trend in McGill score indicating symptom improvement was observed for the biweekly group, with a significantly lower score compared to the waitlisted group appearing at month 6. At the end of follow-up at month 6, the biweekly group was estimated to experience a reduction of approximately 3.8 points on the McGill scale compared to the weekly waitlisted group. Fig 3 illustrates

Table 2. Mean and empirical 95% confidence limits estimated for both SF-36P and McGill from 5000 simulations of the fitted GEE models.

|	Waitlist/Weekly SF-36P (95% CI)	Biweekly SF-36P (95% CI)	Waitlist/Weekly McGill (95% CI)	Biweekly McGill (95% CI)
Baseline	66.1 (60.6 to 71.6)	67.1 (61.2 to 72.9)	29.7 (27.1 to 32.2)	29.7 (27.4 to 32.0)
Month 2—Baseline	61.8 (54.8 to 68.6)	66.9 (61.1 to 72.5)	31.5 (28.9 to 34.1)	29.1 (27.3 to 31.0)
Month 4—Baseline	65.7 (58.4 to 72.8)	67.0 (60.1 to 72.8)	29.3 (26.7 to 31.9)	26.8 (24.6 to 29.1)
Month 6—Baseline	64.3 (57.1 to 71.4)	73.7 (67.7 to 79.8)	29.5 (26.8 to 32.0)	25.9 (23.5 to 29.3)

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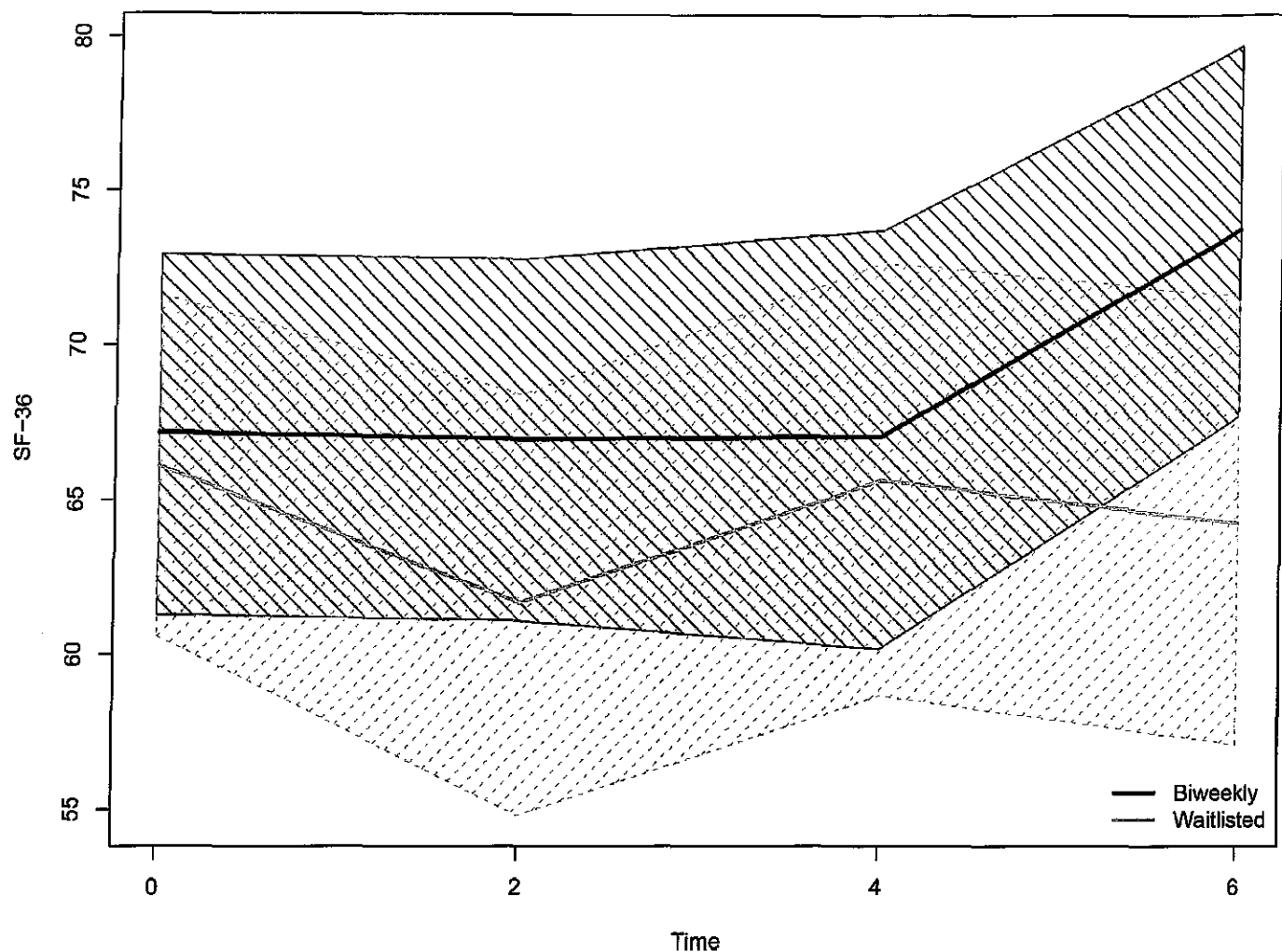
Fig 2. Summary of model-based simulations[xxxiii] of changes in mean SF-36P at 4 measurement timepoints. Scores moving in the positive direction indicate improvement. Scores moving in the positive direction indicate improvement.

the estimated value and 95% confidence intervals. Tables 3 and 4 offer estimates and accompanying statistical values from the GEE modeling of the two outcomes.

Participants reported high usability of acupuncture with 96% of the veterans (averaged across both groups and over all time points) reporting confidence in recommending acupuncture to a friend or family member (or at least a 3 on a five point scale from "Very Confident" to" Not Confident"), 98% reporting that the acupuncture experience was at least pleasant (or at least a 3 on a five point scale from "Extremely Pleasant" to "Extremely Unpleasant"), 97% reporting that their relationship with their practitioner was pleasant (or at least a 3 on a five point scale from "Extremely Pleasant" to "Extremely Unpleasant") and 96% reported that the acupuncture treatments were logical for them (or at least a 3 on a 6 point scale of "Very Logical" to "Not Logical at All"). The trial had only two adverse events: (1) subject in biweekly treatment group reported pain on needling, (2) subject in weekly treatment group reported suicidal thoughts, which study staff followed up with additional medical oversight.
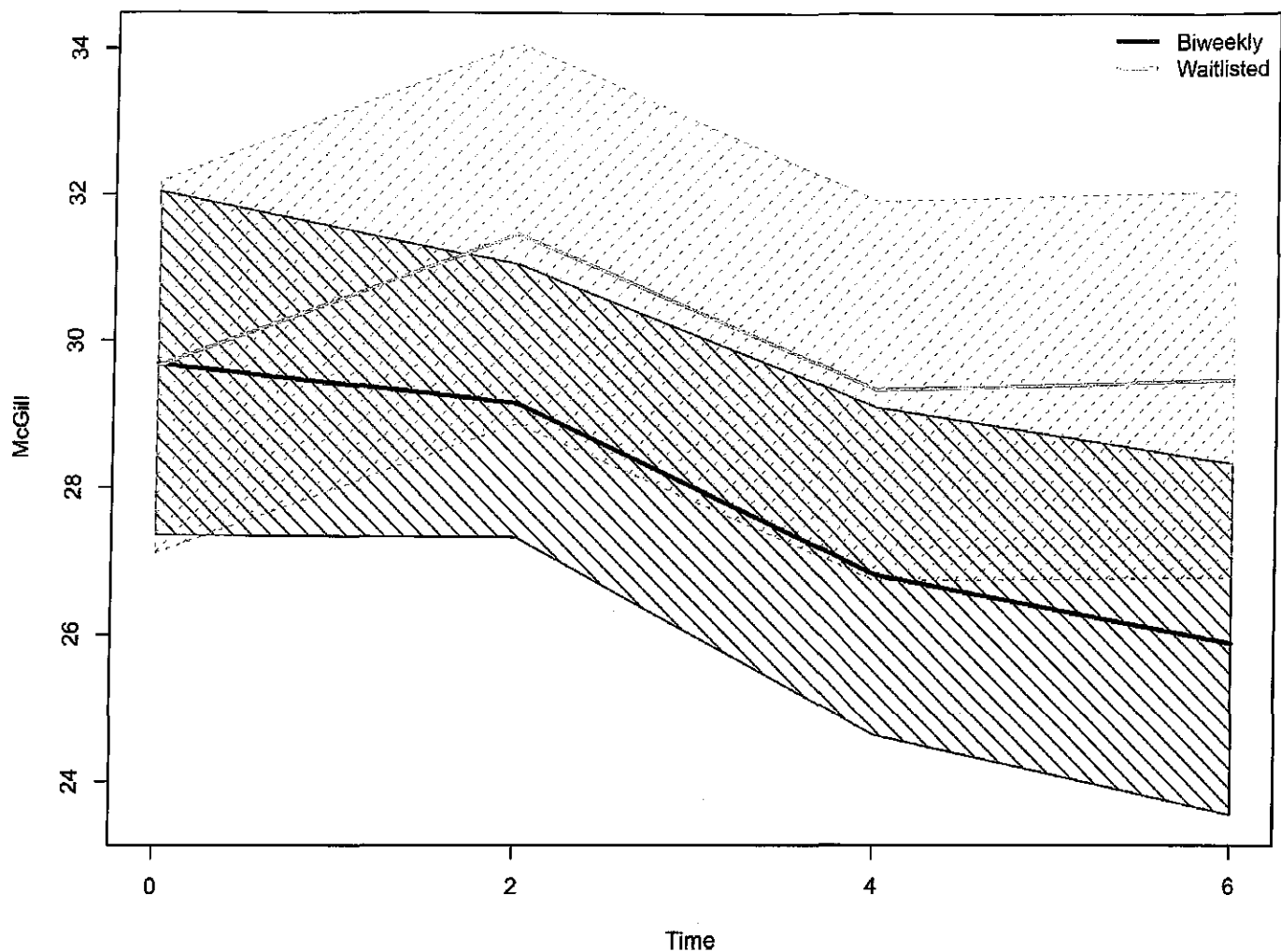
Fig 3. Summary of model-based simulations[xxxiii] of changes in mean McGill Pain Scale at 4 measurement timepoints. Scores moving in the negative direction indicate improvement. Scores moving in the negative direction indicate improvement.

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Table 3. Estimates and accompanying statistical values from the GEE modeling of the 6-month outcome SF-36P.

SF-36	Estimate	Std Err	95% CI	Wald X2	p-value
Intercept	106.91	6.96	(93.27, 120.56)	235.90	<0.001
Biweekly	0.98	4.07	(-7.00, 8.97)	0.06	0.81
Month 2	-4.38	2.52	(-9.32, 0.56)	3.02	0.08
Month 4	-0.42	2.97	(-6.24, 5.40)	0.02	0.89
Month 6	-1.78	3.27	(-8.19, 4.62)	0.30	0.59
Baseline pain	-1.37	0.21	(-1.79, -0.95)	41.12	<0.001
(Biweekly) X (Month 2)	4.18	3.56	(-2.81, 11.16)	1.37	0.24
Biweekly * Month 4	0.33	4.14	(-7.79, 8.46)	0.01	0.94
Biweekly * Month 6	8.39	3.76	(1.03, 15.76)	4.99	0.03

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Table 4. Estimates and accompanying statistical values from the GEE modeling of the outcome McGill Pain scale.

McGill	Estimate	Std Err	95% CI	Wald X2	p-value
Intercept	29.65	1.28	(27.15, 32.15)	539.82	<0.001
Biweekly	0.02	1.73	(-3.38, 3.42)	0.00	0.99
Month 2	1.82	1.06	(-0.26, 3.89)	2.93	0.09
Month 4	-0.31	1.41	(-3.07, 2.45)	0.05	0.82
Month 6	-0.20	1.35	(-2.84, 2.44)	0.02	0.88
Biweekly * Month 2	-2.35	1.29	(-4.88, 0.19)	3.29	0.07
Biweekly * Month 4	-2.56	1.81	(-6.11, 0.99)	2.00	0.16
Biweekly * Month 6	-3.59	1.71	(-6.94, -0.25)	4.43	0.04

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Discussion

This study supports the use of individualized acupuncture treatments for the management of GWI symptoms. Our results are in concordance with numerous other studies indicating that acupuncture is a widely available, safe, effective, and cost-effective option for the treatment of other diseases and syndromes with similar presentations to GWI [35] with high usability in veteran populations [36]. Given this research, it is likely that acupuncture treatment may be an effective, safe, low-cost treatment option for our returning military as well as civilian populations impacted by chronic multi-symptom illness and its co-morbidities.

The mechanisms of acupuncture in the treatment of GWI are unknown, which supports our choice of a low-constraint design. This naturalistic RCT includes individualized protocols, a clinically supported length and dose of treatment, and a wait list control. Data from our wait list arm (Table 2) indicates that symptoms are stable, as has been shown in published 5- and 10- year follow-ups [1,5]. The design aspect of the wait list group eventually receiving weekly acupuncture offers us data to begin to answer questions of minimal dose and satisfies ethical concerns allowing all subjects to receive treatment during the study. A sham acupuncture control arm was not used due to published indications that such sham interventions are effective and thus not appropriate controls; very high quality evidence of this is now available [37,38].

We chose a pragmatic design, and used practitioners in the community, to facilitate adherence and test the use of extant practitioners. Our positive results support the referral of GWI veterans to acupuncture treatments. Our low side effect rate mirrors that of the published literature that acupuncture is safe when provided by professionally trained practitioners [39]. Serious adverse events are extremely rare. In a systematic review of 12 prospective studies scrutinizing over a million treatments, the very low risk of serious adverse event, mostly trauma from needle puncture or infection, was estimated at 0.05 per 10,000 treatments, a risk below that of many common medical treatments [40]. Acupuncture is well tolerated, safe and effective in the management of Gulf War Syndrome. The inclusion of acupuncture in the routine management of this intractable condition is warranted.

Limitations and Future Directions

Our results suggest that 2 months of biweekly acupuncture is not sufficient to affect the outcome of physical function (as measured by the SF-36P), but pain scores (as measured by the McGill Pain Scale) did show group improvement as early as the first follow-up (2 months). These findings underscore the need for more dosage studies to determine the most therapeutic level of treatment for different illness presentations. Currently, we are conducting secondary data analyses exploring the effectiveness of acupuncture treatment on different subtypes of GWI to help treatment providers apply the best protocols for this complex illness. The team is

also categorizing the most effective treatment protocols from a Traditional Chinese Medicine point of view and matching these with different biomedical symptom presentations. Our low-constraint/non-standardized design allowed for collection of naturalistic clinical data, which may increase data validity and make such communications across medical systems more useful.

Other items related to observation window are also of interest. For example, the obvious yet non-statistically significant decrease in Sf-36P component scores which happened while the veterans were waiting for treatment could be natural history but probably is not as observations over longer periods show symptom stability over time [41]. Most likely, this symptom change is due to veterans' frustration in having to wait for treatment; a few of the veterans mentioned this frustration along with an acknowledgment that they were informed of the necessity for a wait list design and knowledge that they would receive treatment at 2 months. We will explore other changes which occurred during the wait list in later analyses as well as the symptom changes associated with different doses of acupuncture.

Although we did find support for our 6-month hypothesis we did not achieve the sample size determined to give us 80% power to see an effect. This leaves us more vulnerable to Type II (false negative) error. Future work should include larger sample sizes to protect against this. Larger sample sizes may also more easily support the use of standardized protocols, which become possible with the implementation of effectiveness trials such as ours that gather a range of clinically relevant treatment options

Supporting Information

S1 File. Protocol.
(DOC)

S2 File. CONSORT Checklist.
(DOC)

S3 File. Example Treatment Protocol for Subject in Biweekly Treatment Condition.
(DOCX)

S4 File. Data Used in Calculations.
(XLSX)

Acknowledgments

Author Contributions

Conceived and designed the experiments: LC MS RS. Performed the experiments: MG KH LC. Analyzed the data: LC TG. Contributed reagents/materials/analysis tools: LC TG. Wrote the paper: LC TG RS MS KH.

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W81XWH-14-1-0533

Dr Lisa Conboy

Appendix B

Statement of Work

<div align="center">

STATEMENT OF WORK – July 9, 2014
PROPOSED START DATE Aug 1, 2014

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Site 1: New England School of Acupuncture 150 California Street Newton, MA 02458 PI: Lisa Conboy	Site #2 Massachusetts General Hospital 55 Fruit St Boston, MA 02114 Consultant: Joe Locascio Statistical Assistance Only

Statement of Work

This project is a secondary data analysis and program evaluation of the Congressionally Directed Medical Research Program funded project "The Effectiveness of Acupuncture in the Treatment of Gulf War Illness" (W81 XWH). Objectives: This application has two objectives to complete using data from the parent grant: 1) Better define and describe this complex disease and how healing may happen using acupuncture, 2) Report on the best acupuncture protocols for GWI veterans and specific GWI presentations. Following the directives of the "Recommendation for Funding" letter of April 11, 2014, two Specific Aims from the original application are supported: **Aim 2: Complete scale construction for remaining psychosocial and clinical measures, Aim 3: Determine the relationships between dose of acupuncture and effect on secondary outcomes in this sample.**

Aim 2: Complete scale construction for remaining psychosocial and clinical measures	Timeline	Site 1 NESA	Site 2 MGH (statistical assistance only)
Major Task 1	Months		
Train research assistants, and review goals with consultants. Once funding is achieved the team will have a face-to-face meeting (using Skype for long-distance members) to review program goals. Follow-up group e-mails will solidify our process. Relevant treatment variables will be determined by our acupuncture expert team members.	1-2	Dr. Conboy	Dr. Locascio
IRB Review. The protocol will be submitted to the IRB as soon as funding is approved. This approval should take no more than a month. Review will take place at our contracted IRB, the New England	1-3	Dr. Conboy	

IRB (www.neirb.com).			
Clean remaining Data. Dr Conboy will execute scale construction of the remaining instruments and create a single database. The RA (with supervision) will pull variables that the team determines to be of relevance from the Acupuncturists' treatment records will be entered onto spreadsheets, defined, tabulated, and categorized.	2-8	Dr. Conboy	
Milestone(s) Achieved: Local IRB/IACUC Approval	3		
Milestone Achieved: HRPO/ACURO Approval	6		
Milestone Achieved: Data cleaned	8		
Aim 3: **Determine the relationships between dose of acupuncture and effect on secondary outcomes in this sample.**			
Major Task 2			
Data analysis: predictors of clinical improvement. Starting in month 9 of the grant Dr. Conboy will perform these statistics with input and direction (statistical assistance) from the study team members Drs Joe Locascio (JL) at Massachusetts General Hospital and Iris Bell (IB) at the University of Arizona.	9-19	Dr. Conboy	Dr. Locascio
Every 6 weeks the consulting team will meet via email or phone to review progress and suggest modifications.	9-19	Dr. Conboy	Dr. Locascio
Our initial assessment of variables will begin with descriptive analyses including simple pair-wise Pearson correlations among our variables. For each type of correlation, both cross-sectional and longitudinal data will be evaluated. Cross-sectional data will be evaluated using only baseline measurements and using the mean of each measure for each participant. Longitudinal associations will be evaluated by correlating participant-specific slope estimates for each measure.	9-12	Dr. Conboy	Dr. Locascio
Exploratory analyses will be used to identify outliers and highly leveraged points, to assess marginal and multivariate normality of outcome	13-15	Dr. Conboy	Dr.

variables and consider data transformations that might improve adherence to model assumptions, and to identify patterns of change over time. Information rich graphical analyses of cross-sectional and longitudinal data will be performed as part of this preliminary exploration of the data.			Locascio
Previous work, our own and that of others, indicates that psychosocial measurements are often highly correlated with each other. Exploratory factor analysis will be used to resolve underlying sources of variation among these measures.	16-19	Dr. Conboy	
Milestone(s) Achieved: Predictors of clinical improvement identified	19	Dr. Conboy	
Major Task 3			
Data analysis: relationship of dose and clinical improvement (month 20-24): Dr Conboy will continue working with the statistical assistance of Dr Joe Locascio, with the input of other team members to determine the relationship between dose of acupuncture and effect on secondary outcomes in the sample. Iris Bell MD will be closely involved to offer advice on modeling and relationships between variables in order to produce a more complete understanding of these relationships in GWI.	20-24	Dr. Conboy	Dr. Locascio
Every 6 weeks the consulting team will meet via email or phone to review progress and suggest modifications.	20-24	Dr. Conboy	Dr. Locascio
Major Task 4			
Final Manuscript write ups. The last 6 months of the project will be used to complete manuscripts and any remaining analyses. The entire study team will be involved.	25-30	Dr. Conboy	Dr. Locascio
Milestone(s) Achieved: Two manuscripts are submitted for publication: (1) *Predictors of Clinical*	30	Dr. Conboy	

Improvement of GWI Symptoms associated with Acupuncture Treatment, (2) The Relationship of Dose of Acupuncture and Clinical Improvement of GWI Symptoms			

Appendix C

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